Published in final edited form as:

Hematol Oncol Clin North Am. 2014 April ; 28(2): 199–216. doi:10.1016/j.hoc.2013.12.003.

Gene Therapy for Hemoglobinopathies:

The State of the Field and the Future

Shanmuganathan Chandrakasan, MD^a and Punam Malik, MD^{b,c,*}

^aDivision of Hematology, Oncology and Bone Marrow Transplant, Cancer and Blood Disease Institute (CBDI), Cincinnati Children's Hospital Medical Center (CCHMC), 3333 Burnet Avenue, Cincinnati, OH 45229, USA

^bDivision of Experimental Hematology/Cancer Biology, Cincinnati Children's Research Foundation, Cancer and Blood Institute (CBDI), Cincinnati Children's Hospital Medical Center (CCHMC), 3333 Burnet Avenue, Cincinnati, OH 45229, USA

^cDivision of Hematology, Cincinnati Children's Research Foundation, Cancer and Blood Institute (CBDI), Cincinnati Children's Hospital Medical Center (CCHMC), 3333 Burnet Avenue, Cincinnati, OH 45229, USA

Keywords

Hemoglobinopathy; Thalassemia; Sickle cell disease; Gene therapy

INTRODUCTION

Over the last 2 decades significant advances have been made in gene therapy for hemoglobinopathies. Gene therapy has exploited the ability of retrovirus (RV) vectors, which are equipped with the machinery to reverse transcribe their RNA into complementary DNA (cDNA) and integrate this cDNA into the host cell genome to deliver therapeutic genes into cells. Inherited hematopoietic disorders are potentially targetable, because hematopoietic stem cells (HSC) can be readily isolated from bone marrow or mobilized peripheral blood, manipulated ex vivo, and transplanted back using current tools and knowledge of bone marrow transplant technology. The initial vectors used for gene therapy were derived from the murine Moloney leukemia virus (MLV), a simple retrovirus belonging to the γ -retrovirus family, and were termed RV vectors. Despite initial successes in several immunodeficiency disorders, limitations of the RV include their inability to infect the quiescent nondividing HSC, hematopoietic malignancies from inadvertent integration of these vectors near cellular proto-oncogenes, and their activation by the viral promoter enhancer in the vector long terminal repeat (LTR) regions. For hemoglobinopathies, an additional limitation is their inability to carry the large cargo of the globin gene and its regulatory elements, required for high-level expression. These limitations prompted the

development of human immunodeficiency virus (HIV)–based vectors. HIV belongs to the lentivirus (LV) family of retroviruses and hence these vectors are termed LV vectors. LV have several advantages for gene therapy for hemoglobinopathies. They differ from RV in their ability to enter an intact nucleus and integrate into nondividing cells, and hence they transduce HSC efficiently. Their self-inactivating (SIN) design, which removes all viral transcriptional machinery, and their ability to carry a large cargo makes them ideally suited for gene therapy for hemoglobinopathies. Since the first use of gene therapy for adenosine deaminase (ADA) deficiency in 1990, generational changes have made the vector design more effective and safe, advanced molecular tools have made it easier to study the insertional effects of gene transfer. Gene therapy has been shown to be effective in correction of many immunodeficiency disorders such as X-linked severe combined immunodeficiency (X-SCID), Wiskott-Aldrich syndrome (WAS), and chronic granulomatous disease (CGD). However, gene therapy for hemoglobinopathies has a unique set of challenges.

Hemoglobinopathies have the additional challenge of requiring high levels of expression of β -/ γ -globin genes for therapeutic correction. Identification of critical regulatory elements needed for high expression of β -globin transgene has made gene therapy for β -hemoglobinopathies (sickle cell disease [SCD] and β -thalassemia) a feasible option. More recently, encouraging results from the first successful gene therapy for a patient with hemoglobin E- β -thalassemia in a French trial has opened up gene therapy as a potential definitive treatment option for patients with β -hemoglobinopathies. Trials with different iterations of the β -globin and γ -globin genes in SIN LV to treat thalassemia or SCD are beginning in Italy and 4 centers in the United States (New York, Memphis, TN; Cincinnati, OH; and Los Angeles, CA).

Because expression of increased levels of fetal hemoglobin (HbF) significantly ameliorates the phenotype of both SCD and β -thalassemia, critical elements involved in γ -globin repression in postnatal life have been identified, which has opened up new gene therapy approaches to increase endogenous γ -globin production without the need to add the globin genes and the large locus control region regulatory elements.

Even more promising is the development of gene editing approaches. The design of zinc finger nuclease (ZFN)–mediated repair was the first such approach. This approach was soon superseded by transcription activator–like effector nucleases (TALENs) and, more recently, by the CRISPR/Cas technology, both facilitating efficient gene editing to correct the genetic defect in situ in cells with high specificity and efficiency. Once this technology is refined, it will allow genetic correction of the β -hemoglobinopathies in situ in HSC, without the need for gene insertion therapy. This article reviews the advances in gene therapy for hemoglobinopathies, with specific reference to β -thalassemia and SCD.

RATIONALE

Disorders of β -globin such as β -thalassemia and SCD are among the most common monogenic defects in the world. Based on World Health Organization worldwide estimates, about 275,000 babies with SCD and 56,000 babies with β -thalassemia are born each year.¹

Improved supportive care has prolonged the survival of these patients. However, there is still significant long-term mortality and morbidity associated with these diseases.

In β -thalassemia, mutations affecting every step in β -globin expression have been reported, from initiation of transcription to posttranslational modification.² These defects either result in decreased β -globin production (β^+) or an absence of β -globin production (β^0). The decreased β -globin synthesis in β -thalassemia results in excess of alpha globin, leading to intracellular precipitation of excess unbound alpha globin chains in erythroid precursors.² This condition results in red cell membrane damage, early cell death, and ineffective erythropoiesis.² At present, lifelong regular transfusions and iron chelation is the mainstay in the management of thalassemia. Despite aggressive iron chelation, iron overload leading to tissue damage and organ toxicity is the leading cause of morbidity and mortality in these patients.^{3,4} Inadequate transfusions lead to massive erythroid hyperplasia, extramedullary hematopoiesis, splenomegaly, and failure to thrive.

SCD is caused by a point mutation (A-T) in the sixth codon of the β -globin gene. This genetic defect results in defective β -globin, which polymerizes in the deoxygenated state leading to change in shape of an otherwise round, doughnut-shaped, flexible red blood cell (RBC) into a hook/sickle-shaped, inflexible RBC that obstructs micro-vessels and has reduced survival from repeated cycles of sickling and unsickling. SCD is characterized clinically by varying degree of anemia, and episodic vaso-occlusive crisis leading to multiorgan damage and premature death. Medical management options currently available for SCD include supportive management of vaso-occlusive crisis, long-term transfusions to avoid or prevent recurrence of severe complications of SCD such as stroke or acute chest syndrome, and HbF induction with hydroxyurea. Despite improvement in the supportive care, the estimated life expectancy for patients with SCD is significantly foreshortened; the median age at death was 42 years for men and 48 years for women with SCD.⁵

At present, allogeneic hematopoietic stem cell transplant (HCT) is the only established definitive curative option for patients with β -thalassemia and SCD. In both these diseases, the overall survival following matched sibling donor (MSD) HCT is excellent in children, with disease-free survival rates greater than 80%, and a graft failure rate of about 10%.^{6,7}

However, most patients do not have a human leucocyte antigen (HLA)-matched sibling. In SCD, it is estimated that less than 14% have a matched sibling and, in the United States,⁸ only 30% of African Americans have an identifiable matched unrelated donor (MUD) for HCT (Fig. 1). Over the last decade, MUD HCT have increasingly been used for management of thalassemia and SCD.⁹ Results from the Italian multicenter trial showed an excellent overall survival and thalassemia-free survival of 97% and 80%, respectively, in low-risk groups. However, in the high-risk groups, the results were less encouraging; overall survival and thalassemia-free survival were considerably lower at 65% and 54%, respectively.¹⁰ Most of this mortality was attributed to grade II to IV graft-versus-host disease (GVHD) or graft failure.¹¹⁻¹³

In addition, there is poor acceptance of allogeneic HCT as a treatment options among patients with SCD and fewer than 400 transplants have been performed for this disease,

because the HCT-related side effects are not acceptable to this patient population (see Fig. 1).^{14,15} Considering all these limitations with allogeneic HCT, gene transfer using autologous stem cells can potentially cure both thalassemia major and SCD, and could overcome the problems in allogeneic HCT, especially lack of available donors and immunologic side effects such as GVHD or graft rejection.

PREREQUISITES FOR SUCCESSFUL GENE THERAPY IN β -HEMOGLOBINOPATHIES

The following requirements need to be met for successful gene therapy for β -globinopathies:

1. High-efficiency gene transfer and high HSC engraftment
2. Position independent or consistent expression independent of the site of integration
3. High level expression of the β -/ γ -globin gene expression
4. Erythroid lineage-specific and developmental stage-specific expression of the transferred β -globin gene
5. Safe expression with little or no risk of insertional mutagenesis/oncogenesis

UNDERSTANDING THE DEVELOPMENTAL SWITCH OF β -GLOBIN AND ITS REGULATION IN POSTNATAL LIFE

In humans, β -like gene cluster is on chromosome 11 and it consists of 5 functional genes: ϵ , $G\gamma$, $A\gamma$, δ , and β .² During different stages of human development, timed switch in expression occurs within this gene cluster. In fetal life, the γ -globin gene (resulting in HbF; $\alpha_2\gamma_2$) is the predominant gene expressed in β -globin locus and β -globin gene expression is repressed. However, after birth, expression of γ -globin gene decreases to negligible levels, with concomitant increase in β -globin expression, which decreases HbF with a corresponding increase in HbA ($\alpha_2\beta_2$). This process of switching from γ - to β -globin is complex, and it is regulated by multiple transcription factors such as GATA1, BCL11A, KLF1, MYB, and SOX6, and chromatin modification by histone deacetylase 1 and 2 (HDAC 1 and 2).^{16,17} The current interest to increase γ -globin expression stems from some important observations. In both SCD and β -thalassemia, hereditary persistence of HbF (HPFH) leads to milder disease phenotype. As a result, pharmacologic measures to induce HbF have resulted in clinical improvement.^{18,19} An in-depth understanding of the role of these regulatory elements in γ -globin gene silencing during postnatal life can help design targeted interventions to reactivate endogenous γ -globin expression.^{16,17}

The regulation of β -globin is also complex, and elements apart from the β -globin promoter are needed for high levels of expression of the β -globin gene.^{20,21} A regulatory element of 40 to 60 Kb upstream of the β -globin gene, known as the locus control region (LCR), is a strong erythroid-specific enhancer that is necessary for high levels of expression of the β -globin gene.²² The LCR is extremely sensitive to DNase 1 in erythroid cells, with 5 DNase hypersensitive (HS) sites, HS1 to HS5 (Fig. 2). Of the 5 HS regions, HS2 was critical for the position independent activity of the LCR, and HS3 was an important determinant of its enhancer activity.^{23,24} Similar to the LCR for the β -globin locus, HS40, a DNase-

hypersensitive site was identified as an alpha globin enhancer 40 Kb upstream of the alpha globin locus acts as an alpha globin enhancer.²⁵

INTRODUCTION TO GENE THERAPY

Many viral and nonviral vectors have been tried for gene transfer into cells. Nonviral vectors include plasmids or naked DNA that require physical means such as electro-poration to enter cells. The gene transfer efficiency is best with virus vectors, because they have evolved over millions of years to be able to readily enter cells. In addition, physical techniques are too harsh for fragile cells such as HSC. Although nanoparticles that can carry the genetic material in a phospholipid scaffold are a promising proposition, a lot of work is still needed to improve their efficiency and specificity.

Most of the viral vectors in preclinical studies and in clinical trials are derived from Retroviridae, a family of viruses that can permanently integrate into cellular genomes. Other viral vectors include adenovirus, herpes simplex virus, vaccinia, pox virus, and adeno-associated virus, but lack the integration apparatus. The viruses in the Retroviridae family that are currently used in clinical trials or in advanced preclinical testing include retrovirus (RV), LV, and foamy virus (FV) vectors. These viral vectors are bio-engineered viral particles in which the genetic elements needed for pathogenicity and replication are removed and are replaced by the cellular transgene of interest. By using these viral vectors, clinicians could use the viral machinery to enter cells and accomplish the delivery and permanent integration of the transgene of interest into human genome, thus correcting the underlying genetic defect (Fig. 3). A synopsis of common terminology used in gene therapy and comparison of different characteristics of the commonly used gene therapy vectors in hemoglobinopathies are given in Tables 1 and 2 respectively.

RV vectors were the first vectors to be used in clinical trials. The vectors had intact viral LTRs, which contain strong ubiquitous enhancers at either end, with some packaging elements and the transgene of interest (Fig. 4). Although these LTR-intact vectors mediated high levels of transgene expression leading to clinical improvement, the success in the trials were soon marred by safety concerns from insertional oncogenesis from transactivation of cellular oncogenes by the RV LTR.²⁶ The lympho-proliferation and leukemia in X-SCID and WAS was ascribed to insertion activation of the LMO2 oncogene.^{27,28} In the gene therapy trial for CGD, after initial success, there was silencing of transgene expression caused by methylation of the viral promoter, and myelodysplasia developed with monosomy 7 as a result of insertional activation of ecotropic viral integration site 1 (EVII/MDS).²⁹

Bioengineering of HIV-1 devoid of any pathogenic elements resulted in the development of lentivirus (LV) vectors. Initial studies had established LV vectors as dependable vehicles for high-efficiency gene transfer. Later, a SIN LV vector was constructed by deleting the viral promoter/enhancer in the U3 region of the 3' LTR, a deletion that gets copied to the 5' LTR, minimizing transactivation of neighboring cellular promoters and creating an improved safety profile.³⁰ In addition, several other advantages of LV were noted compared with RV vectors:

1. LV vector can infect quiescent, nondividing cells including HSC, providing an important advantage because long-term HSC are more quiescent than short-term HSC and precursors.^{30,31} Gene transfer in long-term quiescent HSC offers a stable long-term correction.
2. Because of higher transduction efficiency, shorter culture time of 24 to 48 hours is need for LV-based vectors compared with more than 72 hours for RV vectors.
3. LV vectors can stably carry larger and more complex transgene cassettes with introns and regulatory elements, necessary for high globin gene expression.
4. In experimental systems, they have a safer integration profile: unlike RV vectors, which have an insertion bias for promoters and 5' regulator regions of cellular genes, LV vectors usually insert within the introns of cellular genes, avoiding the regulatory elements near the transcription start site. Insertional dysregulation caused by abnormal splicing of endogenous cellular genes was noted with LV-based gene therapy experiments.^{32,33} LCR-containing LV vectors had approximately 200-fold lower transforming potential compared with the conventional RV vectors.³³ Addition of insulator elements resulted in a further reduction in transforming activity of LCR-containing LV.³³ Thus, based on SIN LV design and supportive data, gene therapy with LV vectors is currently considered to have a lesser risk of insertional oncogenesis than RV vectors and higher efficacy at transferring genes into HSC.

INITIAL VECTOR DEVELOPMENT FOR β -THALASSEMIA

A decade long investigation with β -globin RV vectors resulted in: a) variable and low level expression of the β -globin transgene,^{20,34} b) unstable vectors with multiple rearrangements,³⁵ and c) vectors with low titers.^{36,37} In addition, it was found that only HS2 and HS3 elements of β -globin LCR were most important for expression and 3' HS could be removed without affecting expression.^{23,24} Vectors were made containing only core elements of HS2, HS3, and HS4 of the LCR to accommodate the transgene cassette into RV.^{38,39} Another important observation was wide variation in gene expression, dependent on the site of integration.³⁹ These chromatin position effects led to the discovery and use of chromatin insulator elements, such as chicken hypersensitive site 4 (cHS4), into RV vectors.^{40,41} Insulators are boundary elements flanking the gene cluster of interest and protect the transgene from silencing, resulting in position-independent expression, and have enhancer blocking activity.⁴¹ The prototypic cHS4 insulator has 2 cores: a proximal 250-bp core element⁴² and distal 400-bp element in which all the insulator activity of cHS4 resides.⁴³

INITIAL DEVELOPMENT OF LV-BASED VECTORS FOR β -THALASSEMIA

Long-term correction of thalassemia with an LV vector in a murine thalassemia intermedia model was first reported by May and colleagues,⁴⁴ with an average increase in hemoglobin by 3 to 4 g/dL per LV vector copy and correction of red cell indices. LV vector-mediated correction of the thalassemia phenotype was also reported in other thalassemia mouse models.^{45,46} However, in these studies multiple vector copies were needed for phenotypic

correction because of variable transgene expression.^{45,46} Further optimization of the LCR components was done by different investigators, which included larger HS2 to HS4 elements of LCR, incorporation of cHS4 insulator elements,⁴⁷ or the inclusion of HS1.⁴⁸ Later, correction of human β^0 -thalassemia cells was shown in human erythropoiesis models in vitro and in a xenograft model.⁴⁹ LV vectors carrying a mutated β -globin gene [that confers it with antisickling properties (β^{T87Q})⁵⁰] or a γ -globin gene were shown to be effective in corrections of both thalassemia and SCD in murine models.⁵¹

HUMAN GENE THERAPY FOR THALASSEMIA

The basic steps of gene therapy for hemoglobinopathies are shown in Fig. 5. In June 2007, the first successful gene therapy for a transfusion-dependent hemoglobin (Hb) $\beta^E\beta^0$ -thalassemia was done using an SIN LV-based β^{T87Q} vector.⁵² A transduction efficiency of about 30% was reported. The patient received myeloablative busulfan conditioning followed by infusion of 3.9×10^6 CD34+ cells/kg of gene-transduced HSC. After engraftment he had a gradual increase in gene-marked cells to 10% to 20%, and became transfusion independent with a stable hemoglobin of 8.5 to 9 g/dL by 2 years after gene transfer. Although the thalassemia phenotype was partially corrected with gene therapy there was an initial safety concern. Insertional site analysis showed clonal expansion at the high-mobility-group AT-hook 2 (HMGA2) locus in erythroid cells to 10% to 12%. However, the clone stabilized and, at 5 years after gene therapy, it contributed only 2% to 3% of the circulating nucleated cells, and the patient is still transfusion independent.⁵³ In addition, instability of tandem cHS4 core elements was noted in some cells. To date, 1 more patient has been enrolled in this protocol, but the outcome is not known.

In the United States, a group led by Sadelain and colleagues⁵⁴ started a phase 1 to 2 β -thalassemia gene therapy trial in 2011; so far 2 patients have been reported to have received gene-corrected CD34+ cells, and their outcome is awaited.

GENE THERAPY FOR SCD

Successes in β -thalassemia gene therapy led to similar studies in SCD. Both γ -globin-based and modified β -globin-based vectors have been developed for SCD gene therapy. Synthetic β -globin (β^{T87Q} and β^{AS3}) with antisickling properties has been developed by 2 different groups.^{50,55} By making 3 amino acid substitutions to the normal human β -globin gene (T87Q, E22A, and G16D), Townes and colleagues⁵⁵ bio-engineered a recombinant β -globin that prevents axial and lateral contacts in the sickle polymer. The recombinant β -globin designated β^{AS3} inhibits sickle hemoglobin polymerization in the deoxygenated state, and also provides a competitive advantage compared with sickle β -globin for interaction with the α globin.⁵⁵

Pawliuk and colleagues⁵⁰ were the first to show correction of the SCD phenotype in a murine model by expressing β^{T87Q} with an LV vector. Later, Levasseur and colleagues⁵⁶ used a SIN LV vector carrying antisickling globin β^{AS3} to correct the phenotype in sickle mice with human α and β^s globin. The study also showed the feasibility of high gene transfer efficiency in HSC with long-term persistent expression without cytokine prestimulation. Romero and colleagues⁵⁷ recently used LV-based vector encoding β^{AS3} to

show consistent expression of modified β -globin and reversal of RBC physiologic changes in red cells differentiated for CD34+ bone marrow stem cells derived from a patient with SCD.

Because γ -globin is known to have antisickling properties, gene therapy using γ -globin was considered. However, in adult life, the γ -globin transcripts are highly silenced; to circumvent this, γ -globin driven by β -globin promoters and enhancers were developed.^{58,59} Pestina and colleagues⁵⁸ showed that the γ -globin gene driven by 3.1 kb of β -globin LCR and a 130-bp β -globin promoter resulted in increase in Hb by 2.6 g/dL/vector copy (VC) and amelioration of disease symptoms. In addition, replacement of γ -globin 3'-untranslated region (3'-UTR) with the β -globin 3'-UTR resulted in a higher Hb increment of 4.1 g/dL/VC.⁵⁸ Our group substituted the exons of γ -globin gene for β -globin in the β -globin vector and showed phenotypic correction in the humanized sickle mouse model.⁵⁹ In addition, by using reduced intensity conditioning and variable cell dose, we showed that HbF exceeding 10% and a stable chimerism of 20% gene-modified HSCs in the marrow results in phenotypic correction.⁵⁹ To date, no patient with SCD has been reported to be enrolled in clinical gene therapy trials, although our group has just opened a gene therapy trial for SCD in Cincinnati.

RECENT ADVANCES

Role of Mobilizing Agents to Achieve Adequate Stem Cell Dose

To achieve transfusion independence, an adequate number of gene-transduced HSCs have to be given. At present, G-CSF mobilized CD34+ and also bone marrow-derived HSCs have been used in gene therapy trials. Specific concerns with G-CSF mobilization in patients with thalassemia include marked leukocytosis and thrombocytosis, and risk for potential thrombotic events in splenectomized patients.⁶⁰ In addition, in patients with SCD, the use of G-CSF is contraindicated because there was a high incidence of mortality associated with its use.⁶¹ Plerixafor (AMD3100) has been found to safely mobilize an adequate amount of CD 34+ cells in patients with thalassemia.⁶² In addition, when plerixafor was used in combination with G-CSF, it resulted in a more primitive HSC phenotype with a high reconstitution potential and enrichment of CD34+ cell concentration in the BM.⁶³

In Vivo Selection: Giving Survival Advantage to Transduced Stem Cells

One of the limitations of β -thalassemia gene therapy in human is to achieve good gene-marked HSC engraftment. Efforts have been made to identify ways to give selective survival advantage to gene-transduced HSCs. Zhao and colleagues⁶⁴ developed an LV vector encoding both human γ -globin and an enzyme, methylguanine methyltransferase (MGMT). The addition of MGMT rendered transduced cells resistant to cytotoxic effects of alkylating agents. Thus, when exposed to alkylating agents either before or after transplantation, survival advantage was shown in transduced HSCs compared with the untransduced HSCs.⁶⁴ By this approach they were able to achieve a higher number of γ -globin-expressing red cells and HbF, leading to resolution of anemia.⁶⁴ Although this approach has translational potential, exposure to additional alkylating agents is less likely to be tolerated in patients with SCD and thalassemia.

Newer Vectors

Although the safety of gene therapy has potentially increased by the use of SIN LV vectors, on integration site analysis they still insert in transcriptionally active sites. Newer vectors with potentially better safety profiles have been developed, of which FV-based vectors have shown promising preclinical results.

FV-based vectors have several unique advantages compared with existing RV-based and LV-based vectors (see Table 2). (1) FV is nonpathogenic; (2) FV tends to integrate into nongenic regions predominantly; and (3) it has the largest retrovirus genome compared with RV and LV, hence can be used to package a larger transgene cassette. However, the disadvantage is that, like RV, they require cell division before integration. Morianos and colleagues⁶⁵ recently showed that FV vectors with the α -globin HS40 regulatory element resulted in stable expression of human β -globin gene in erythroid cell lines, murine model of thalassemia, and erythroid cells derived from gene-transduced human CD34+ cells from patients with thalassemia.

Transcriptional Manipulation to Increase HbF

Hydroxyurea and other agents have been used to induce HbF in patients with SCD and thalassemia. The exact mechanism of how these medications cause an increase in HbF is still unclear. In recent years novel manipulation approaches have been used to activate the endogenously silent γ -globin expression to increase HbF levels. Recent reports have highlighted the role of KLF1 and BCL11A in repression of γ -globin expression in adult life.^{66,67} Several proof-of-concept studies to suppress the level of expression of KLF1 and BCL11A by using short-hairpin RNA in erythroid progenitors and conditional knock-down in murine models have shown increase in γ -globin expression resulting in increased HbF without significantly affecting the erythropoiesis.^{66–68} Apart from repression of transcription factors, another approach used for increasing the production of endogenous γ -globin is by activation of its promoter.⁶⁹ By using artificial zinc finger transcriptional activation factor, Wilber and colleagues⁶⁹ showed an increase in HbF in erythroblasts derived from human CD34+ cells.

At present, the dual approach of combining activation of endogenous γ -globin with either gene therapy using γ -globin vector or inducers of HbF such as 5-azacytidine have been tried.⁶⁸ Initial results are promising: if successful, this could result in high levels of HbF leading to potential therapeutic benefit in patients with SCD and β -thalassemia.

INDUCED PLURIPOTENT STEM CELL AND GENE EDITING APPROACH

Advances in induced pluripotent stem cells (iPSC) and gene editing technologies has offered a promising opportunity to correct genetic defects using autologous somatic cells. iPSC offer the potential for an endless supply of stem cells for gene manipulation and correction strategies.^{70,71} iPSC are generated from mature somatic cells by forced reprogramming of genes to induce the repressed multilineage differentiation potential. The major advantage of iPSC in a setting of gene therapy is that it offers the possibility to screen and choose the ideal clone with safe integration and high transgene expression profile. Feasibility of this approach was recently shown by using an LV-mediated β -globin gene transfer in iPSC from

patients with β -thalassemia.⁷² The principles of gene editing and the use of iPSC are discussed below.

The ability to make targeted double-stranded DNA breaks (DSB) by using different site-directed endonuclease approaches have been exploited by researchers to correct the defects in the target genes. ZFNs,⁷³ transcription activator–like effector nucleases (TALENs),⁷⁴ and clustered regulatory interspaced short palindromic repeat (CRISPR/Cas) endonucleases⁷⁵ systems have been used to make site-specific DSBs. Following DSBs, the breaks in DNA are repaired by 2 mechanisms: nonhomologous end joining (NHEJ) and homology-directed repair (HDR)/homologous recombination. NHEJ mediates direct joining of the DNA ends in a DSB, which leads to a high incidence of insertion or deletion at the break site. However, in HDR, a homologous sequence is used as a template for generation of the missing DNA sequence at the break point. Despite the high fidelity of the HDR, the frequency of its occurrence is low. When site-directed DSB is combined with facilitated homologous recombination using a donor repair DNA element, the frequency of HDR could be significantly improved (Fig. 6). The ZFN system was the first of these systems to be established, and feasibility studies done in a human iPSC line has shown promising initial results.

ZFN-mediated correction of α -thalassemia has been shown in human iPSC.⁷⁶ Proof-of-concept studies have been reported using ZFN and TALEN for correction of SCD. Sebastiano and colleagues⁷⁷ reported in situ correction of SCD mutation in 2 iPSC cell lines derived from patients with sickle cell anemia; by this approach they reported mean target efficacy of correction to be 9.8%. However, the frequency of successful recombination is still low enough to warrant drug selection approaches, making it more complex and difficult to work with in somatic stem cells.⁷⁷

Due to limited modularity of ZFN and low specificity leading to off-target effects, research has shifted to a more open platform with high specificity and modularity such as TALENs.⁷⁸ Sun and colleagues⁷⁹ recently showed correction of SCD mutation in iPSC cells with highly specific TALEN in combination with *piggyBack* transposon. At present, there are limited tools available to measure the off-target effects of the endonuclease-induced DSBs. Although the gene editing approach is promising, a lot of work is still needed in improving the specificity and efficiency of gene editing. Simultaneous advances in iPSC to generate long-term HSC could help realize the potential of gene editing approaches in correcting genetic defects in these disorders.

References

1. Modell, B. Global epidemiology of haemoglobin disorders and derived service indicators. London, UK: World Health Organization; -2008.p. 417-96.
2. Weatherall, DJ. The thalassemias: disorders of globin synthesis. In: Kaushansky, K.; Lichtman, M.; Beutler, E., editors. Williams hematology. 8. United States: McGraw-Hill; 2010.
3. Musallam KM, Cappellini MD, Taher AT. Iron overload in beta-thalassemia intermedia: an emerging concern. *Curr Opin Hematol.* 2013; 20:187–92. [PubMed: 23426199]
4. Olivieri NF, Nathan DG, MacMillan JH, et al. Survival in medically treated patients with homozygous beta-thalassemia. *N Engl J Med.* 1994; 331:574–8. [PubMed: 8047081]

5. Platt OS, Brambilla DJ, Rosse WF, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. *N Engl J Med*. 1994; 330:1639–44. [PubMed: 7993409]
6. Shenoy S. Hematopoietic stem-cell transplantation for sickle cell disease: current evidence and opinions. *Ther Adv Hematol*. 2013; 4:335–44. [PubMed: 24082994]
7. Angelucci E. Hematopoietic stem cell transplantation in thalassemia. *Hematology Am Soc Hematol Educ Program*. 2010; 2010:456–62. [PubMed: 21239835]
8. Walters MC, Patience M, Leisenring W, et al. Stable mixed hematopoietic chimerism after bone marrow transplantation for sickle cell anemia. *Biol Blood Marrow Transplant*. 2001; 7:665–73. [PubMed: 11787529]
9. Mentzer WC, Heller S, Pearle PR, et al. Availability of related donors for bone marrow transplantation in sickle cell anemia. *Am J Pediatr Hematol Oncol*. 1994; 16:27–9. [PubMed: 8311169]
10. La Nasa G, Argioli F, Giardini C, et al. Unrelated bone marrow transplantation for beta-thalassemia patients: the experience of the Italian Bone Marrow Transplant Group. *Ann N Y Acad Sci*. 2005; 1054:186–95. [PubMed: 16339665]
11. Goussetis E, Peristeri I, Kitra V, et al. HLA-matched sibling stem cell transplantation in children with beta-thalassemia with anti-thymocyte globulin as part of the preparative regimen: the Greek experience. *Bone Marrow Transplant*. 2012; 47:1061–6. [PubMed: 22080966]
12. Fleischhauer K, Locatelli F, Zecca M, et al. Graft rejection after unrelated donor hematopoietic stem cell transplantation for thalassemia is associated with nonpermissive HLA-DPB1 disparity in host-versus-graft direction. *Blood*. 2006; 107:2984–92. [PubMed: 16317094]
13. La Nasa G, Giardini C, Argioli F, et al. Unrelated donor bone marrow transplantation for thalassemia: the effect of extended haplotypes. *Blood*. 2002; 99:4350–6. [PubMed: 12036861]
14. Thompson AL, Bridley A, Twohy E, et al. An educational symposium for patients with sickle cell disease and their families: results from surveys of knowledge and factors influencing decisions about hematopoietic stem cell transplant. *Pediatr Blood Cancer*. 2013; 60:1946–51. [PubMed: 23900784]
15. Walters MC, Patience M, Leisenring W, et al. Barriers to bone marrow transplantation for sickle cell anemia. *Biol Blood Marrow Transplant*. 1996; 2:100–4. [PubMed: 9118298]
16. Sankaran VG, Orkin SH. The switch from fetal to adult hemoglobin. *Cold Spring Harb Perspect Med*. 2013; 3:a011643. [PubMed: 23209159]
17. Sankaran VG. Targeted therapeutic strategies for fetal hemoglobin induction. *Hematology Am Soc Hematol Educ Program*. 2011; 2011:459–65. [PubMed: 22160074]
18. Musallam KM, Taher AT, Cappellini MD, et al. Clinical experience with fetal hemoglobin induction therapy in patients with beta-thalassemia. *Blood*. 2013; 121:2199–212. quiz: 372. [PubMed: 23315167]
19. Voskaridou E, Christoulas D, Bilalis A, et al. The effect of prolonged administration of hydroxyurea on morbidity and mortality in adult patients with sickle cell syndromes: results of a 17-year, single-center trial (LaSHS). *Blood*. 2010; 115:2354–63. [PubMed: 19903897]
20. Cone RD, Weber-Benarous A, Baorto D, et al. Regulated expression of a complete human beta-globin gene encoded by a transmissible retrovirus vector. *Mol Cell Biol*. 1987; 7:887–97. [PubMed: 3029570]
21. Karlsson S, Papayannopoulou T, Schweiger SG, et al. Retroviral-mediated transfer of genomic globin genes leads to regulated production of RNA and protein. *Proc Natl Acad Sci U S A*. 1987; 84:2411–5. [PubMed: 3470803]
22. Grosveld F, van Assendelft GB, Greaves DR, et al. Position-independent, high-level expression of the human beta-globin gene in transgenic mice. *Cell*. 1987; 51:975–85. [PubMed: 3690667]
23. Collis P, Antoniou M, Grosveld F. Definition of the minimal requirements within the human beta-globin gene and the dominant control region for high level expression. *EMBO J*. 1990; 9:233–40. [PubMed: 2295312]
24. Ellis J, Tan-Un KC, Harper A, et al. A dominant chromatin-opening activity in 5' hypersensitive site 3 of the human beta-globin locus control region. *EMBO J*. 1996; 15:562–8. [PubMed: 8599939]

25. Jarman AP, Wood WG, Sharpe JA, et al. Characterization of the major regulatory element upstream of the human alpha-globin gene cluster. *Mol Cell Biol.* 1991; 11:4679–89. [PubMed: 1875946]
26. Cavazzana-Calvo M, Fischer A, Hacein-Bey-Abina S, et al. Gene therapy for primary immunodeficiencies: part 1. *Curr Opin Immunol.* 2012; 24:580–4. [PubMed: 22981681]
27. Hacein-Bey-Abina S, Garrigue A, Wang GP, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest.* 2008; 118:3132–42. [PubMed: 18688285]
28. Boztug K, Schmidt M, Schwarzer A, et al. Stem-cell gene therapy for the Wiskott-Aldrich syndrome. *N Engl J Med.* 2010; 363:1918–27. [PubMed: 21067383]
29. Stein S, Ott MG, Schultze-Strasser S, et al. Genomic instability and myelodys-plasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. *Nat Med.* 2010; 16:198–204. [PubMed: 20098431]
30. Miyoshi H, Blomer U, Takahashi M, et al. Development of a self-inactivating lenti-virus vector. *J Virol.* 1998; 72:8150–7. [PubMed: 9733856]
31. Naldini L, Blomer U, Gage FH, et al. Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. *Proc Natl Acad Sci U S A.* 1996; 93:11382–8. [PubMed: 8876144]
32. Hargrove PW, Kepes S, Hanawa H, et al. Globin lentiviral vector insertions can perturb the expression of endogenous genes in beta-thalassemic hematopoietic cells. *Mol Ther.* 2008; 16:525–33. [PubMed: 18195719]
33. Arumugam PI, Higashimoto T, Urbinati F, et al. Genotoxic potential of lineage-specific lentivirus vectors carrying the beta-globin locus control region. *Mol Ther.* 2009; 17:1929–37. [PubMed: 19707188]
34. Dzierzak EA, Papayannopoulou T, Mulligan RC. Lineage-specific expression of a human beta-globin gene in murine bone marrow transplant recipients reconstituted with retrovirus-transduced stem cells. *Nature.* 1988; 331:35–41. [PubMed: 2893284]
35. Novak U, Harris EA, Forrester W, et al. High-level beta-globin expression after retroviral transfer of locus activation region-containing human beta-globin gene derivatives into murine erythroleukemia cells. *Proc Natl Acad Sci U S A.* 1990; 87:3386–90. [PubMed: 2333288]
36. Plavec I, Papayannopoulou T, Maury C, et al. A human beta-globin gene fused to the human beta-globin locus control region is expressed at high levels in erythroid cells of mice engrafted with retrovirus-transduced hematopoietic stem cells. *Blood.* 1993; 81:1384–92. [PubMed: 8443396]
37. Chang JC, Liu D, Kan YW. A 36-base-pair core sequence of locus control region enhances retrovirally transferred human beta-globin gene expression. *Proc Natl Acad Sci U S A.* 1992; 89:3107–10. [PubMed: 1557419]
38. Leboulch P, Huang GM, Humphries RK, et al. Mutagenesis of retroviral vectors transducing human beta-globin gene and beta-globin locus control region derivatives results in stable transmission of an active transcriptional structure. *EMBO J.* 1994; 13:3065–76. [PubMed: 8039501]
39. Sadelain M, Wang CH, Antoniou M, et al. Generation of a high-titer retroviral vector capable of expressing high levels of the human beta-globin gene. *Proc Natl Acad Sci U S A.* 1995; 92:6728–32. [PubMed: 7624311]
40. Sun FL, Elgin SC. Putting boundaries on silence. *Cell.* 1999; 99:459–62. [PubMed: 10589674]
41. Chung JH, Bell AC, Felsenfeld G. Characterization of the chicken beta-globin insulator. *Proc Natl Acad Sci U S A.* 1997; 94:575–80. [PubMed: 9012826]
42. Recillas-Targa F, Pikaart MJ, Burgess-Beusse B, et al. Position-effect protection and enhancer blocking by the chicken beta-globin insulator are separable activities. *Proc Natl Acad Sci U S A.* 2002; 99:6883–8. [PubMed: 12011446]
43. Arumugam PI, Urbinati F, Velu CS, et al. The 3' region of the chicken hypersensitive site-4 insulator has properties similar to its core and is required for full insulator activity. *PLoS one.* 2009; 4:e6995. [PubMed: 19746166]
44. May C, Rivella S, Callegari J, et al. Therapeutic haemoglobin synthesis in beta-thalassaemic mice expressing lentivirus-encoded human beta-globin. *Nature.* 2000; 406:82–6. [PubMed: 10894546]

45. Rivella S, May C, Chadburn A, et al. A novel murine model of Cooley anemia and its rescue by lentiviral-mediated human beta-globin gene transfer. *Blood*. 2003; 101:2932–9. [PubMed: 12480689]
46. Persons DA, Hargrove PW, Allay ER, et al. The degree of phenotypic correction of murine beta - thalassemia intermedia following lentiviral-mediated transfer of a human gamma-globin gene is influenced by chromosomal position effects and vector copy number. *Blood*. 2003; 101:2175–83. [PubMed: 12411297]
47. Arumugam PI, Scholes J, Perelman N, et al. Improved human beta-globin expression from self-inactivating lentiviral vectors carrying the chicken hypersensitive site-4 (cHS4) insulator element. *Mol Ther*. 2007; 15:1863–71. [PubMed: 17622240]
48. Lisowski L, Sadelain M. Locus control region elements HS1 and HS4 enhance the therapeutic efficacy of globin gene transfer in beta-thalassemic mice. *Blood*. 2007; 110:4175–8. [PubMed: 17921347]
49. Puthenveetil G, Scholes J, Carbonell D, et al. Successful correction of the human beta-thalassemia major phenotype using a lentiviral vector. *Blood*. 2004; 104:3445–53. [PubMed: 15292064]
50. Pawliuk R, Westerman KA, Fabry ME, et al. Correction of sickle cell disease in transgenic mouse models by gene therapy. *Science*. 2001; 294:2368–71. [PubMed: 11743206]
51. Persons DA, Allay ER, Sabatino DE, et al. Functional requirements for phenotypic correction of murine beta-thalassemia: implications for human gene therapy. *Blood*. 2001; 97:3275–82. [PubMed: 11342459]
52. Cavazzana-Calvo M, Payen E, Negre O, et al. Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. *Nature*. 2010; 467:318–22. [PubMed: 20844535]
53. Payen E, Leboulch P. Advances in stem cell transplantation and gene therapy in the beta-hemoglobinopathies. *Hematology Am Soc Hematol Educ Program*. 2012; 2012:276–83. [PubMed: 23233592]
54. Boulad, F. Gene therapy for hemoglobinopathies BMT tandem meeting; Salt Lake City. February 13–17, 2013;
55. Levasseur DN, Ryan TM, Reilly MP, et al. A recombinant human hemoglobin with anti-sickling properties greater than fetal hemoglobin. *J Biol Chem*. 2004; 279:27518–24. [PubMed: 15084588]
56. Levasseur DN, Ryan TM, Pawlik KM, et al. Correction of a mouse model of sickle cell disease: lentiviral/antisickling beta-globin gene transduction of unmobilized, purified hematopoietic stem cells. *Blood*. 2003; 102:4312–9. [PubMed: 12933581]
57. Romero Z, Urbinati F, Geiger S, et al. Beta-globin gene transfer to human bone marrow for sickle cell disease. *J Clin Invest*. 2013; 123(8):3317–30.
58. Pestina TI, Hargrove PW, Jay D, et al. Correction of murine sickle cell disease using gamma-globin lentiviral vectors to mediate high-level expression of fetal hemoglobin. *Mol Ther*. 2009; 17:245–52. [PubMed: 19050697]
59. Perumbeti A, Higashimoto T, Urbinati F, et al. A novel human gamma-globin gene vector for genetic correction of sickle cell anemia in a humanized sickle mouse model: critical determinants for successful correction. *Blood*. 2009; 114:1174–85. [PubMed: 19474450]
60. Taher AT, Otrrock ZK, Uthman I, et al. Thalassemia and hypercoagulability. *Blood Rev*. 2008; 22:283–92. [PubMed: 18511163]
61. Adler BK, Salzman DE, Carabasi MH, et al. Fatal sickle cell crisis after granulocyte colony-stimulating factor administration. *Blood*. 2001; 97:3313–4. [PubMed: 11368061]
62. Yannaki E, Papayannopoulou T, Jonlin E, et al. Hematopoietic stem cell mobilization for gene therapy of adult patients with severe beta-thalassemia: results of clinical trials using G-CSF or plerixafor in splenectomized and nonsplenectomized subjects. *Mol Ther*. 2012; 20:230–8. [PubMed: 21952171]
63. Yannaki E, Karponi G, Zervou F, et al. Hematopoietic stem cell mobilization for gene therapy: superior mobilization by the combination of plerixafor plus GCSF in patients with thalassemia major. *Hum Gene Ther*. 2013; 24(10):852–60. [PubMed: 24001178]
64. Zhao H, Pestina TI, Nasimuzzaman M, et al. Amelioration of murine beta-thalassemia through drug selection of hematopoietic stem cells transduced with a lentiviral vector encoding both

- gamma-globin and the MGMT drug-resistance gene. *Blood*. 2009; 113:5747–56. [PubMed: 19365082]
65. Morianos I, Siapati EK, Pongas G, et al. Comparative analysis of FV vectors with human alpha- or beta-globin gene regulatory elements for the correction of beta-thalassemia. *Gene Ther*. 2012; 19:303–11. [PubMed: 21734726]
 66. Zhou D, Liu K, Sun CW, et al. KLF1 regulates BCL11A expression and gamma-to beta-globin gene switching. *Nat Genet*. 2010; 42:742–4. [PubMed: 20676097]
 67. Sankaran VG, Menne TF, Xu J, et al. Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. *Science*. 2008; 322:1839–42. [PubMed: 19056937]
 68. Xu J, Peng C, Sankaran VG, et al. Correction of sickle cell disease in adult mice by interference with fetal hemoglobin silencing. *Science*. 2011; 334:993–6. [PubMed: 21998251]
 69. Wilber A, Tschulena U, Hargrove PW, et al. A zinc-finger transcriptional activator designed to interact with the gamma-globin gene promoters enhances fetal hemoglobin production in primary human adult erythroblasts. *Blood*. 2010; 115:3033–41. [PubMed: 20190190]
 70. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007; 131:861–72. [PubMed: 18035408]
 71. Park IH, Zhao R, West JA, et al. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature*. 2008; 451:141–6. [PubMed: 18157115]
 72. Papapetrou EP, Lee G, Malani N, et al. Genomic safe harbors permit high beta-globin transgene expression in thalassemia induced pluripotent stem cells. *Nat Biotechnol*. 2011; 29:73–8. [PubMed: 21151124]
 73. Urnov FD, Miller JC, Lee YL, et al. Highly efficient endogenous human gene correction using designed zinc-finger nucleases. *Nature*. 2005; 435:646–51. [PubMed: 15806097]
 74. Bedell VM, Wang Y, Campbell JM, et al. In vivo genome editing using a high-efficiency TALEN system. *Nature*. 2012; 491:114–8. [PubMed: 23000899]
 75. Cho SW, Kim S, Kim JM, et al. Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. *Nat Biotechnol*. 2013; 31:230–2. [PubMed: 23360966]
 76. Chang CJ, Bouhassira EE. Zinc-finger nuclease-mediated correction of alpha-thalassemia in iPS cells. *Blood*. 2012; 120:3906–14. [PubMed: 23002118]
 77. Sebastiano V, Maeder ML, Angstman JF, et al. In situ genetic correction of the sickle cell anemia mutation in human induced pluripotent stem cells using engineered zinc finger nucleases. *Stem Cells*. 2011; 29:1717–26. [PubMed: 21898685]
 78. Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. *Nat Rev Mol Cell Biol*. 2013; 14:49–55. [PubMed: 23169466]
 79. Sun N, Zhao H. Seamless correction of the sickle cell disease mutation of the HBB gene in human induced pluripotent stem cells using TALENs. *Biotechnol Bioeng*. 2013 Epub ahead of print.

KEY POINTS

- Hemoglobinopathies are the most common genetic defects worldwide. Hematopoietic stem cell (HSC) transplant, although curative, is limited by the availability of matched donors.
- Genetic modification of autologous HSC overcomes the availability of donors (every patient is their own donor) and immunological side effects (graft versus host disease/graft rejection).
- Critical determinants unique to gene therapy for hemoglobinopathies are erythroid-lineage and developmental stage-specific high levels of transgene expression and pretrans-plant conditioning that will allow 10%–20% gene-modified HSC chimerism.
- Scientific insights into regulation of the globin gene locus and improvements in gene transfer technology have led to development of β -/ γ -globin based additive gene therapy a clinical reality.
- Clinical trials with β -/ γ -globin lentivirus vectors are now open at multiple sites and transfusion independence following gene therapy has been reported in 1 patient with β -thalassemia.
- Promising new technologies such as induced pluripotent stem cells and genome editing can usher in a new era in the field of gene therapy.

Fig. 1.
The frequency of donors and transplants in patients with SCD.

Fig. 2.

The human γ -globin gene locus on chromosome 11, showing the ontology of expression of the embryonic, fetal, and adult globin genes, controlled by the LCR. In adult life, the transcription of γ -globin is highly repressed. Some of the major transcription factors involved in the repression of γ -globin are highlighted.

Fig. 3.

The production and life cycle of a gene therapy lentivirus vector. The desired viral vectors are made in packaging cells with genetic elements provided separately by different plasmid vectors carrying *gag* (capsid and matrix proteins) and *pol* (protease, reverse transcriptase, and integrase), *VSV-G* envelope, and *Rev* (nuclear export protein). The viral vector is harvested in the supernatant, concentrated and/or purified, and quantified. When the viral vector is added to the medium with HSC, it binds and fuses with the cell membrane of HSC and single-stranded RNA genome is released into the cytoplasm and converted to double-stranded cDNA by the vector reverse transcriptase. The double-stranded DNA forms a preintegration complex that enters the nucleus, where the vector integrase integrates it randomly in the genome of the host cell.

Fig. 4.

Gene therapy viruses and vectors. The genome of both wild-type (A) murine MLV and (B) the HIV-1 virus and the gene therapy vectors derived from them. The initial RVs had full-length LTRs with intact U3 region (which carries the viral enhancer and promoter). With the current generation of LVs, the U3 region of the 3' LTR is deleted, and in the 5' LTR, it is replaced by a cytomegalovirus (CMV) promoter in the 5' LTR. The CMV promoter is only used in packaging the vector, and is not transmitted to host cells. The HIV envelope is replaced by VSV-G envelope. (C) A prototypic globin LV vector is shown.

*- To be used if the gene transduced cells fails to engraft
 #- High dose chemotherapy such as Busulfan

Fig. 5.

Steps of gene transfer into humans. Autologous G-CSF mobilized peripheral blood HSC or bone marrow HSC are harvested and isolated to a pure population. The HSC are transduced for 24 to 48 hours with the gene therapy vector. Conditioning chemotherapy is given concurrently to open up HSC niche. The gene-transduced HSC are infused intravenously.

Zinc finger nucleases (ZFNs)
Transcription activator–like effector nucleases (TALENs)
RNA-guided nuclease - CRISPR/Cas system

Fig. 6.

Gene editing approach. Targeted DSB are made at the site of genetic defect using different customized site-specific endonucleases (ZFN/TALEN/CRISPR/Cas). Following DSB, a donor template strand with correct genome sequence is introduced, which facilitates homologous repair leading to correction of the underlying genetic defect. If the donor template is not provided, the DSB is repaired by NHEJ.

Table 1

Synopsis of commonly used gene therapy terminology

Viral vector	Bioengineered genetically disabled viral particles devoid of the components responsible for pathogenic potential but that act as carriers of the therapeutic transgene of interest
LTR	Long terminal repeats are identical sequences at the ends of RVs: LTR contains U3, R, and U5 regions. The U3 region has strong promoter and enhancer activity and is used by the virus to reverse transcribe and integrate the genetic material into the host genomes
SIN	Self-inactivating vector design is used to improve safety. Deletion of the U3 region in the 3' LTR deletes the TATA box and enhancer and abolishes the LTR promoter and enhancer activity. This deletion does not affect vector integration
Transgene	Human cellular gene (transgene) carried by the vector
Promoter	Cellular promoter needed for transgene expression
Enhancer	Cellular enhancers are short segments of DNA that enhance the transcription of the cellular gene, unlike promoters they may not be in close proximity to the gene
Insulator	Boundary elements flanking the gene cluster of interest; they protect the transgene from silencing by surrounding heterochromatin. Insulators also have enhancer blocking activity
Process	
Titers	Vectors are made from different plasmid systems in packaging cells. The vector yield or "titer" is measured as the number of particles per milliliter
Transduction efficiency	Percentage of cells that have been genetically modified by the vector. The higher the transduction (gene transfer) efficiency the better the outcome
Insertion site	The site in the host chromosome where the vector integrates the transgene with minimal viral elements. The preference of insertion sites vary among different integrating vectors
Vector copy	Number of transgene/vector copies integrated per cell of interest

Table 2

Properties of gene therapy vectors used in hemoglobinopathies

	Gamma RV Vectors	LV Vectors	FV Vectors
Genus	Retroviridae	Retroviridae	Retroviridae
Viral genome	RNA	RNA	RNA → cDNA
Pathogenicity	Derived from pathogenic murine leukemia virus	Derived from pathogenic HIV virus	Derived from nonpathogenic, nonhuman FV
Cell division requirement	Yes	No	Yes
Packaging limitation	Up to 5 Kb, unstable with large transgene cassettes	Up to 7 Kb	Up to 9 Kb, large transgene cassettes
Transduction efficiency	High in dividing cells	High in dividing and nondividing cells	Efficient entry in dividing and nondividing cells. However, integration occurs in dividing cells
Integration site	Preferentially near transcription start sites (60%–70%)	Preferentially within transcribed genes (60%–70%)	Integration into nongenic regions is preferred approximately 60% of the time. However, integrants that occur in gene-rich regions have the same profile as RV
Safety in clinical trials	Leukemia in X-SCID and WAS trials MDS/monosomy 7 in CGD trial	No overt malignancy seen after 2–6 years Clonal expansion of HMGA2 in one thalassemia patient, that has now decreased	No human clinical data
Experimental genotoxicity data	High risk for insertion oncogenesis	Safer than RV. Safety high with self-inactivating design and use of cellular promoters and enhancers	Safer integration profile than RV and LV
Main advantages	Longest human data and experience SIN RV vector design may address safety concerns	Better safety profile than RV vectors Can stably carry larger cargo Efficiently transduce nondividing HSC	Nonpathogenic, can carry a large cargo and may be safer than RV and LV
Main disadvantage	Clinically significant risk of insertion oncogenesis Lower capacity Does not transduce non-dividing cells	Might induce dysregulation/disruption of cellular genes, albeit at low frequency	Still not in human trials