Review article

Gene Therapy in Thalassemia and Hemoglobinopathies

Laura Breda¹, Roberto Gambari² and Stefano Rivella¹

¹ Weill College Medical Center, Department of Pediatrics, Division of Hematology-Oncology, NY, USA.
² Department of Biochemistry and Molecular Biology, Section of Molecular Biology, University of Ferrara, Italy

Correspondence to: Prof Stefano Rivella, Weill College Medical Center, Department of Pediatrics – Division of Hematology-Oncology, 515 E 71st street, S702, 10021 New York, NY, USA. Tel: +212 746 4941, Fax: +212 746 8423, e-mail: str2010@med.cornell.edu

Published: November 13, 2009
Received: October 12, 2009
Accepted: November 12, 2009

Medit J of Hemat Infect Dis 2009, 1(1): e2009008 DOI 10.4084/MJHID.2009.008
This article is available from: http://www.mjhid.org/article/view/5089
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract: Sickle cell disease (SCD) and ß-thalassemia represent the most common hemoglobinopathies caused, respectively, by the alteration of structural features or deficient production of the β-chain of the Hb molecule. Other hemoglobinopathies are characterized by different mutations in the α- or β-globin genes and are associated with anemia and might require periodic or chronic blood transfusions. Therefore, ß-thalassemia, SCD and other hemoglobinopathies are excellent candidates for genetic approaches since they are monogenic disorders and, potentially, could be cured by introducing or correcting a single gene into the hematopoietic compartment or a single stem cell. Initial attempts at gene transfer of these hemoglobinopathies have proved unsuccessful due to limitations of available gene transfer vectors. With the advent of lentiviral vectors many of the initial limitations have been overcome. New approaches have also focused on targeting the specific mutation in the β-globin genes, correcting the DNA sequence or manipulating the fate of RNA translation and splicing to restore β-globin chain synthesis. These techniques have the potential to correct the defect into hematopoietic stem cells or be utilized to modify stem cells generated from patients affected by these disorders. This review discusses gene therapy strategies for the hemoglobinopathies, including the use of lentiviral vectors, generation of induced pluripotent stem cells (iPS) cells, gene targeting, splice-switching and stop codon readthrough.

β-thalassemia, sickle cell anemia and other hemoglobinopathies: The thalassemias are a group of disorders due to a large number of heterogeneous mutations causing abnormal globin gene expression resulting in the total absence or quantitative reduction of globin chain synthesis. Mutations in the α- or β-globin gene lead to α- and β-thalassemia, respectively. α-Thalassemia is usually due to deletions within the α-globin gene cluster, leading to loss of function of one or both α-globin genes in each locus. However, non-deletion mutations have been described, although they are much less frequent. Depending on the number of genes that are unable to synthesize the α-globin protein, different clinical manifestations can be observed. If one or two α-globin genes are mutated (in cis or...
trans), normally no or minimal hematological effects are seen, and individuals are normally silent thalassemia carriers or show α-thalassemia trait1. If three out of four genes are mutated, the condition is called hemoglobin H (HbH) disease, resulting in a hemolytic anemia that can worsen with febrile illness or exposure to certain drugs, chemicals, or infectious agents. Hemoglobin H disease is characterized by moderate to severe anemia, hepatosplenomegaly, and jaundice. Transfusion may occasionally be required and, if provided frequently, can lead to iron overload. If all four α-globin genes are deleted, the resulting condition is called α-thalassemia major, which is so severe that death occurs in utero. Children rescued through intrauterine transfusions remain dependent on red blood cell transfusions for survival1.

The thalassemias are characterized by their clinical severity and genetic mutations. Patients with Cooley’s anemia, also known as β-thalassemia major, which is the most severe form of this disease, require many blood transfusions per year and is characterized by ineffective erythropoiesis and extra medullary hematopoiesis (EMH)1. If untreated, β-thalassemia major is fatal in the first few years of life1. In β-thalassemia intermedia, where a greater number of β-globin chains are synthesized, the clinical picture is milder, and the patients require only infrequent or no transfusions8,11. In both thalassemias, with time the spleen is enlarged, the hemoglobin level decreases, and progressive iron overload occurs from increased GI iron absorption in addition to transfusions1. The vast majority of β-thalassemias are caused by point mutations within the gene or its immediate flanking sequences and are classified according to the mechanism by which they affect gene regulation: transcription, RNA processing and mRNA translation1. These mutations are also classified as β0 and β+ according to the quantity of β-globin chains synthesized. Mutations that lead to alternative splicing are associated with reduced synthesis of normal β-globin mRNA and protein and are defined β−. In contrast, mutations that completely impair β-globin synthesis (for instance premature termination codons or PTCs) are defined β0. Depending on the association of these different mutations, patients are classified into three principal groups with none, very low or low β-globin production (β0/0, 0/+; +/+ respectively). The levels of fetal hemoglobin (HbF) account for a large part of the clinical heterogeneity observed in patients with β-thalassemia. Variation in HbF expression among individuals is an inheritable disease modifier and high HbF (composed from 2 α- and 2 γ-chains) levels generally correlate with reduced morbidity and mortality in this disorder, since the γ-globin chains combine with the excess α-chains.

A single mutation leads to SCD, causing an adenine (A) to thymidine (T) substitution in codon 6 (GAG-GTG), which leads to insertion of valine in place of glutamic acid in the β-globin chain. The resulting Hb (HbS) has the unique property of polymerizing when deoxygenated1. When the polymer becomes abundant, the red cells “sickle”, stiff rods form that stretch and distort the red cells. These distorted cells can obstruct blood flow through the small vessels, and the restricted oxygen delivery to the tissues damages cells, injures organs, and produces pain. Similarly to SCD, other hemoglobinopathies can be triggered by the substitution of one amino acid (HbE5,6,2), deletion of a portion of the amino acid sequence (Hb Gun Hill1), abnormal hybridization between two chains (Hb Lepore8,9), or abnormal elongation of the globin chain (Hb Constant Spring10). These abnormal Hbs can have a variety of pathophysiologically significant effects, including ineffective erythropoiesis and anemia1.

SCD and the thalassemias are quite common among Asian, African, African-American and Mediterranean populations1. It has been estimated that approximately 7% of the world population are carriers of such disorders, and that 300,000–400,000 children with severe forms of these diseases are born each year11,17.

**Hematopoietic stem cell transplantation:** Current disease management of β-thalassemia consists of prenatal diagnosis, transfusion therapy, or allogeneic BMT11-13. Only the latter is potentially curative14. The first successful BMT of β-thalassemia was reported in 198215. Consequently, several centers have utilized this approach as definitive therapy16-18. The most extensive experience in treating β-thalassemia patients with BMT is that of Lucarelli and coworkers in Italy18. Established protocols can lead to a high success of thalassemia-free survival, although the transplant-related mortality is still significant and the chronic graft-versus-host disease is still a potential long-term complication of allogeneic HSCs transplantation17,19. In addition, availability of allogeneic bone marrow is limited by finding an identical human leucocyte antigen (HLA) matched bone marrow donor. However, development of new techniques to improve the management of graft-versus-host disease, to perform BMT from
unrelated donors and cord blood stem cells may expand the pool of potential donors in the near future.20

In addition, patients with severe β-thalassemia and SCD might benefit from new genetic and cellular approaches. From this perspective, β-thalassemia and SCD are excellent candidate diseases for genetically based therapies in autologous hematopoietic stem cells (HSCs)21-23. Alternatively, somatic cells reprogrammed to induced pluripotent stem cells might also provide a possible new approach to treat β-thalassemia24,25.

**Gene transfer using oncoretroviral vectors:** Gene addition mediated by retroviral vectors is an attractive approach for monogenic disorder. However, when applied to hemoglobinopathies, this strategy raises major challenges in terms of controlling transgene expression, which should be erythroid-specific, elevated, position independent and sustained over time. In fact, many studies were performed before positive preclinical data were generated. The first attempts were done using oncoviruses. These viruses belong to the large family of Retroviridae and are characterized by a genome that encodes the genes gag-pol and env26. Onco-retroviral vectors, such as those derived from Moloney murine leukemia virus, efficiently transfer therapeutic genes into murine hematopoietic stem cells (HSC) without transferring any viral gene.27 Recombinant oncoretroviruses were the first viral vectors used to transfer the human β-globin gene in mouse HSCs28,29. These experiments resulted in tissue-specific but low and variable (position-dependent) human β-globin expression in bone marrow chimeras, usually varying between 0 and 2% of endogenous mouse β-globin mRNA levels29,30-33. Studies aimed at increasing expression levels of transferred β-globin genes have focused on including locus control region (LCR) elements of the human β-globin gene locus into oncoretroviral vectors. The LCR contains cis-acting DNase I hypersensitivity sites (HS) that are critical for high-level, long-term, position-independent, and erythroid-specific expression.34,35 These HS elements contain several DNA-binding motifs for transcriptional and chromatin remodeling factors that facilitates chromatin opening. Also, these genomic regions allow for binding of other regulatory elements required for high-level expression of the β-globin gene36. Incorporation of the core elements of HS2, HS3, and HS4 of the human β-globin LCR significantly increased expression levels in murine erythroleukemia (MEL) cells but failed to abolish positional variability of expression.37,35 Additional efforts aimed to include larger elements resulted in the inability of the vector to incorporate large quantities of genetic material, as shown by the rearrangements of the transferred sequences38-41. Since these rearrangements frequently occur because of activation of splicing sites of the LCR sequence contained in the retroviral RNA, additional attempts were done to eliminate these sites. However, even these new vectors failed to include HS elements sufficient large to considerably increase expression of the β-globin gene.37,35

Additional erythroid-specific transcriptional elements were investigated within oncoretroviral vectors, including the HS40 regulatory region from the human α-locus42-44 and alternative promoters. The promoter of ankyrin, a red cell membrane protein, has shown some promise in transgenic mice and in transduced MEL cells45. In mice, the ankyrin promoter has been used to drive expression of the human γ-globin gene resulting, at double copy, in an average expression of 8% of that of the endogenous α-globin genes46. To overcome transcriptional silencing of the γ-globin promoter in hematopoietic chimeras, mutant γ-globin promoters from patients with hereditary persistence of fetal hemoglobin (HPFH) were also investigated47,48. The Greek mutation at position −117 thus appeared to substantially increase γ-globin expression in MEL cells47. However, even these vectors failed to increase the level of the β-globin gene to therapeutic levels.

Although oncoretrovirus vectors integrate into the genome, many integrants undergo transcriptional silencing, posing an additional challenge to the success of gene therapy using these vectors. Kalberer and co-workers attempted to avoid gene silencing by preselecting ex vivo retrovirally transduced hematopoietic stem cells on the basis of expression of the green fluorescent protein (GFP). In this vector the GFP gene was driven by the phosphoglycerate kinase promoter, while the human β-globin gene by its own promoter and small elements from the LCR48. Using this approach, in vivo hematopoietic stem cell gene silencing and age-dependent extinction of expression were avoided, although suboptimal expression levels and heterocellular position effects persisted.

Another major limitation is that oncoretroviral vectors need to infect cells before and close to their division, otherwise the viral RNA cannot migrate into the nucleus due to the presence of a nuclear
membrane. Since most hematopoietic stem cells are in a quiescent state, they must be induced with cytokines to divide in order to achieve higher transduction efficiencies and overall expression levels. Stimulation of quiescent hematopoietic stem cells, however, impairs or halts their long-term repopulating capacities.

**Gene Transfer Using Lentiviral Vectors:** With the extensive research on human immunodeficiency virus-1, it has been realized that lentivirus, engineered to be devoid of any pathogenic elements, can become efficient gene transfer vectors. Lentiviruses are characterized by a complex genome that encodes a number of accessory proteins besides the canonical retroviral genes gag-pol and env. They share all the common characteristic of retroviral replication including receptor-mediated entry, capsid uncoating, reverse transcription of the viral RNA, and integration into the host cell genome. In addition, they are able to transduce non-replicating cells, which confers to these viruses a special value for the development of clinically functional gene vectors. Moreover, compared to oncoretroviral vectors, the stabilization of the proviral mRNA genome by the interaction of the accessory protein Rev with its cognate motif Rev-responsive element (RRE), increases their range of application, since larger genomic elements can be introduced in their genome with limited or no sequence rearrangement. Therefore, lentiviral vectors are thus likely to be selected as vectors of choice for the stable delivery of regulated transgenes in stem cell–based gene therapy. The use of lentiviral vectors has allowed the introduction of large genomic elements from the β-globin locus, different promoters, enhancers, and chromatin structure determinants that led to lineage-specific and elevated of β-, γ- and α-globin expression in vivo. This resulted, in the amelioration or correction of anemia and secondary organ damage in several murine models of hemoglobinopathies, making the recombinant lentiviruses the most effective vector system to date for gene therapy of these disorders.

α-Thalassemia could potentially be a target for fetal gene therapy since fetuses with this disorder usually die between the third trimester of pregnancy and soon after birth. The potential use of lentiviral vectors to treat α-thalassemia was investigated a vector containing the HS2, 3, and 4 of the LCR from the human β-globin locus, and the human α-globin gene promoter directing the human α-globin gene. Using this vector, Han and colleagues performed gene delivery in utero during midgestation targeting embryos affected by a lethal form of α-thalassemia. They showed that in newborn mice, the human α-globin gene expression was detected in the liver, spleen, and peripheral blood. The human α-globin gene expression was at the peak at 3–4 months, when it reached 20% in some recipients. However, the expression declined at 7 months. Colony-forming assays in these mice showed low levels of transduction and lack of human α-globin transcript. Thus, lentiviral vectors can be an effective vehicle for delivering the human α-globin gene into erythroid cells in utero, but, in the mouse model, delivery at late midgestation could not transduce hematopoietic stem cells adequately to sustain gene expression.

Treatment of β-thalassemia, SCD and other disorders through lentiviral mediated gene transfer is studied in murine and primate models. The original studies in mice showed that lentiviral mediated human β-globin gene transfer can rescue mice affected by β-thalassemia intermedia and β-thalassemia major. The mouse β-globin cluster has two adult β-globin genes, β\text{minor} and β\text{major} globin. Thalassemic mice were generated with deletion of both the β\text{minor} and β\text{major}-globin on one allele, designated th3/+ mice (63; 64). Also adult th3/+ mice have a degree of disease severity (hepatosplenomegaly, anemia, aberrant erythrocyte morphology) comparable to that of patients affected by β-TI. May and colleagues tested two lentiviral vectors termed RNS1 (carrying minimal core LCR elements) and TNS9 (with large LCR fragments encompassing HS2, HS3 and HS4; approximately 3.2 kb in size) on th3/+ mice. Compared to RNS1, mice recipient of the larger TNS9 vector maintained higher human β-globin transcript levels over time showing amelioration of red cell pathology (anisocytosis and poikilocytosis) and significantly increased hemoglobin levels (from 8-9 g/dL to 11-13 g/dL). The massive splenomegaly found in chimeras engrafted with control th3/+ bone marrow was not observed in TNS9-treated animals. This correction was sustained in secondary mice.

Mice completely lacking adult β-globin genes (th3/th3) die late in gestation, limiting their utilization as a model for Cooley’s anemia. For this reason, adult animals affected by Cooley’s anemia were generated by transplantation of hematopoietic fetal liver cells harvested from th3/th3 embryos at E14.5 into lethally irradiated syngeneic adult recipients. Hematological analyses of engrafted mice performed 6 to 8 weeks post-transplant revealed severe anemia due not to pancytopenia but rather to low red blood cell and...
underwent hypersensitive sites from the ß-globin locus control region in mouse models of ß-thalassemia (68). They showed that genetically corrected erythroblasts exhibited corrected reticulocyte counts and amelioration of Hemoglobin concentration, anisocytosis, and poikilocytosis. Moreover, the proportion of irreversibly sickled cells, SCD-associated splenomegaly, and characteristic urine concentration defect in SCD and BERK mice were vastly improved or corrected by ß87. Using a similar vector, Levasseur and colleagues obtained equivalent results. They transduced Sca1+ c-Kit+Lin− cells rather than unselected bone marrow cells and achieved durable therapeutic results (5–7 months) following transplantation of 100 cells in lethally irradiated C57BL/6 mice13,114.

Samakoglu and coworkers applied the principle of RNA interference (RNAi) to down-regulate the ß-globin mRNA in CD34(+) cells from patients affected by SCD116. They utilized a lentiviral vector harboring a promoterless small-hairpin RNA (shRNA) within the intron of a recombinant γ-globin gene. Expression of both γ-globin and the lariat-embedded small interfering RNA (siRNA) was induced upon erythroid differentiation, specifically downregulating the targeted gene in tissue and differentiation stage-specific fashion. The position of the shRNA within the intron was critical to concurrently achieve high transgene expression, effective siRNA generation and minimal interferon induction.

Miccio and colleagues also utilized an erythroid-specific lentiviral vector driving the expression of the human ß-globin gene from a minimal promoter/enhancer element containing two hypersensitive sites from the ß-globin locus control region in mouse models of ß-thalassemia (68). They showed that genetically corrected erythroblasts underwent in vivo selection. The selected erythroblast that derived from progenitors harboring proviral integrations in genome sites and were more favorable to high levels of vector expression. These data suggested that a regimen of partially myeloablative transplantation might be sufficient to achieve a chimerism that would therapeutic in ß-thalassemic patients.

While correction of murine models of ß-thalassemia has been achieved through lentiviral-mediated high levels of globin gene transfer into mouse HSCs, transduction of human HSCs is less robust and may be inadequate to achieve therapeutic levels of genetically modified erythroid cells. Zhao and coworkers therefore developed a double gene lentiviral vector encoding both human γ-globin under the transcriptional control of erythroid regulatory elements and methylguanine methyltransferase (MGMT), driven by a constitutive cellular promoter60. MGMT is an alkyltransferase that normally functions to repair cellular DNA damage at the O6 position of guanine69,70. The cytotoxic effects of alkylating agents, such as temozolomide and 1,3-bis-chloroethyl-1-nitrosourea (BCNU), can be prevented if there is adequate expression of MGMT, which removes the O6 adduct from the modified DNA. Variant MGMT proteins with specific amino acid changes retain significant activity while possessing the useful property of resistance to inactivation by O6-benzylguanine (BG)71. BG can be used to inactivate endogenous MGMT to enhance the specificity of alkylator-mediated cell death to cells not expressing the variant form. Therefore, expression of these variant forms of MGMT provides cellular resistance to alkylator drugs, which can be administered to kill residual untransduced HSCs, whereas transduced cells are protected. To test this hypothesis, mice transplanted with ß-thalassemic HSCs cells transduced with a lentiviral γ-globin/MGMT vector were treated with BCNU60. This led to significant increas in the number of γ-globin–expressing red cells, the amount of fetal hemoglobin and resolution of anemia. One important advantage of using the γ-globin gene, normally expressed exclusively during fetal life, is that high level γ-globin expression would be therapeutic not only for ß-thalassemia, but also SCD. Interestingly, selection of transduced HSCs was also obtained when cells were drug-treated before transplantation. These data suggest that coexpression of MGMT allowed autologous, γ-globin vector-transduced ß-thalassemic HSCs to be enriched to therapeutic levels through either pre or post-transplantation selection.

Imren and colleagues engrafted immunodeficient mice with human cord blood cells infected with a lentiviral vector encoding an anti-sickling ß-globin transgene15,72. After 6-months, half of the human erythroid and myeloid progenitors regenerated in
the mice containing the transgene, and erythroid cells derived in vitro from these cells produced high levels of the β-globin protein. In addition, these authors investigated the integrated proviral copies showing that 86% of the proviral inserts had occurred within genes, including several genes implicated in human leukemia. These findings indicate effective transduction of very primitive human cord blood cells achieving robust and erythroid-specific production of therapeutically relevant levels of β-globin protein. The frequency of proviral integration within genes observed in this study and the data from Miccio and coworkers that indicate that selected erythroblasts were derived from progenitors harboring proviral integrations more favorable to high levels of vector expression, indicate that regulated hematopoiesis might require additional safety modifications to prevent potential genotoxic effects\(^{79-81}\). This risk is inherent to the integration of foreign genetic material and the risk of insertional oncogenesis has been established both in mice and humans\(^{73-78}\).

In light of these results, genetic elements with enhancer-blocking properties, such as insulators, could increase the safety of the clinical trials. These elements have been investigated to shelter the vector from the repressive influence of flanking chromatin by blocking interactions between regulatory elements within the vector and chromosomal elements at the site of integration\(^{79-81}\). This property of insulators can also be harnessed to diminish the risk that the vector will activate a neighboring oncogene\(^{82,83}\). The initial studies indicated that inclusion of the cHS4 insulator element into the 3' LTR of recombinant murine leukemia virus increases the probability that randomly integrated proviruses will express the transgene\(^{46,84-86}\). Puthenveetil and coworkers tested a lentiviral vector carrying the human β-globin expression cassette flanked by a chromatin insulator in transfection-dependent human β-thalassemia major cells\(^{87}\). Using this vector, they demonstrated normal expression of human β-globin in erythroid cells produced in vitro. They also observed restoration of effective erythropoiesis and reversal of the abnormally elevated apoptosis that characterizes β-thalassemia. The gene-corrected human β-thalassemia progenitor cells were also transplanted into immune-deficient mice, where they underwent normal erythroid differentiation, expressed normal levels of human β-globin, and displayed normal effective erythropoiesis 3 to 4 months after xenotransplantation. Based on all these preclinical studies on mouse models of β-thalassemia and SCD, clinical trials have been proposed or are underway\(^{53}\). Figure 1A depicts this approach.

Alternatively, the homologous recombination pathway can be harnessed to avoid random integration. Zinc-finger nucleases (ZFNs) can been used to enhance the frequency of gene correction\(^{88,89}\). However, achieving the full potential of ZFNs for genome engineering in human cells requires their efficient delivery to the relevant cell types. Lombardo and colleagues exploited the infectivity of integrase-defective lentiviral vectors (IDLV) to express ZFNs and provide the template DNA for gene correction in different cell types\(^{90}\). IDLV-mediated delivery supported high rates (13–39%) of editing at the IL-2 receptor common γ-chain gene (IL2RG) across different cell types as well as human embryonic stem cells (5%), allowing selection-free isolation of clonogenic cells with the desired genetic modification. Therefore, this technique opens new and exciting possibilities. By modifying the ZFN binding specificity and selecting an appropriate donor sequence, one could target the IDLV-ZFN system to any individual site in the human genome avoiding random integration (Figure 1B) and, potentially, genome toxicity\(^{88-91}\).

However, there are current obstacles to successfully apply this therapeutic approach to humans. Some of them include the need for improved efficiency of gene delivery, insertion of the gene into non-oncogenic sites and the potential negative or positive contributions of the β-thalassemic genotype and potential modifiers to the effectiveness of the gene transfer\(^7\). Original studies in animal models utilized mice with deletions of the β-globin genes. These mutations do not reflect the phenotypic variability observed in β-thalassemic patients. Thus, there is a gap in knowledge between our understanding of the primary mutation, the corresponding phenotype, and the approach to cure an individual patient based on his/her genotype (i.e. understanding of the disease and its treatment by genetic modalities). To date this variability has not been addressed and no studies have focused on the efficacy of gene therapy in relation to the different genotypes of the patients. Although gene therapy is an area of active clinical investigation, the aforementioned obstacles limit its use in the management of thalassemia. Nonetheless, as we showed in our review the successful transfer of globin genes into hematopoietic cells of humans has been demonstrated and is encouraging.
Gene Correction and Ips Cells: Triplex-forming oligonucleotides and triplex-forming peptide nucleic acids (PNAs) have been shown to stimulate recombination in mammalian cells via site-specific binding and creation of altered helical structures that provoke DNA repair\textsuperscript{92,93}. Cotransfection of PNAs and recombinatory donor DNA fragments, Chin and co-workers demonstrated that these complexes can promote single base-pair modification at the start of the second intron of the beta-globin gene, the site of a common thalassemia-associated mutation\textsuperscript{94}. This single base pair change was detected by the restoration of proper splicing of transcripts produced from a green fluorescent protein-beta-globin fusion gene. The ability of these PNAs to induce recombination was dependent on dose, sequence, cell-cycle stage, and the presence of a homologous donor DNA molecule. They also showed that these PNAs were effective in stimulating the modification of the endogenous beta-globin locus in human cells, including primary hematopoietic progenitor cells. Enhanced recombination, however, did not alter helical structures superior to 0.4%\textsuperscript{94}. However, this technology could be a powerful tool in combination with the generation of stem cells. In particular, introduction of the genes Oct3/4, Sox2 with either Klf4 and c-Myc or Nanog and Lin28 genes can induced pluripotent stem (iPS) cells\textsuperscript{95,115,24,96}. Ye and coworkers showed that iPS cells can be generated from cells derived from skin fibroblasts, amniotic fluid or chorionic villus sampling of patients with beta-thalassemia\textsuperscript{97}. Subsequently, the iPS cells were differentiated into hematopoietic cells that synthesized hemoglobin. Therefore, in the future the mutation in the beta-globin gene of these iPS cells could be corrected by gene targeting and the cells differentiated into HSCs to be returned to the patient\textsuperscript{94}. Figure 1C depicts this approach. In fact, mice affected by SCD were cured using this strategy\textsuperscript{98}. However, there are some obstacles that need to be overcome before iPS treatment of beta-thalassemia will be utilized. One of the most pressing problems is elimination of the transcription factors when they are no longer needed. Second, it is necessary to reestablish the correct reprogramming so that the iPS cells do not develop into tumors.

Splice-Switching and Stop Codon Readthrough: Defective beta-globin gene expression and beta-globin deficiency can be attributed to almost 200 thalassemic mutations. However, only 10 mutations are responsible for the majority of cases worldwide and some of the most frequent cause aberrant splicing of intron 1 (IVS1-110, IVS1-6, IVS1-5) or intron 2 (IVS2-654, IVS2-745)\textsuperscript{99,112}. These mutations lead to incorrectly spliced mRNAs, even though the correct splice sites remain undamaged and potentially functional. Use of small nuclear RNA (snRNA) and splice-switching oligonucleotides represents a promising approach since these molecules can restore the correct splicing re-establishing the synthesis of the normal protein\textsuperscript{94,100-105}. Therefore blocking the aberrant splice sites with antisense oligonucleotides forces the splicing machinery to reselect the existing correct splice sites. Expression of antisense sequences targeted to the aberrant splice sites in thalassemic pre-mRNA has been successful, restoring the correct splicing pattern and ultimately restoring hemoglobin synthesis\textsuperscript{102,93}. This was demonstrated in HSCs and erythroid progenitor cells from a patient with IVS2-745/IVS2-1.
Anticodon base pairing, inducing a ribosomal read-through in vitro of a splice-switching oligonucleotide, a morpholino oligomer conjugated with an arginine-rich peptide. Repaired β-globin mRNA restored significant amounts of hemoglobin in the peripheral blood of the IVS2-654 thalassemia mouse, improving the number and quality of erythroid cells.

Another approach showing a great potential for the treatment of genetic disorders characterized by premature termination codons (PTCs) is the use of drugs to induce stop codon readthrough. These modified RNA would protected against non-sense mediated mRNA decay (NMD) and allow production of a protein. Aminoglycoside antibiotics can decrease the accuracy in the codon-anticodon base pairing, inducing a ribosomal read-through of premature termination codon. These findings have led to the development of a pharmacologic approach to treat thalassemic patients carrying the 80-39 mutation, which introduces a PTC in codon 39 of the β-globin gene and is one of the most frequent thalassemic mutations in the Mediterranean littoral. Aminoglycosides and analogous molecules were tested in their ability to restore β-globin protein synthesis on human erythroid cells (K562) carrying a lentiviral construct containing the 80-39 globin-gene. Treatment of these cells with genetin (G418) and other aminoglycosides restored the production of β-globin. Moreover, after FACS and high performance liquid chromatography (HPLC) analyses, G418 was also demonstrated to partially correct the biological function of the β0-39 globin mRNA in erythroid precursor cells from β0-39 homozygous thalassemia patients. This study strongly suggests that ribosomal read-through should be considered a novel approach for treatment of β0 thalassemia caused by premature stop codon mutations and NMD.

References

1. Adamkiewicz TV, Szaboecs P, Haigh A, Baker KS, Staba S, Kedar A, Chiang KY, Krishnamurti L, Boyer MW, Kurtzberg J, Wagner JE, Wingard JR, Yeager AM (2007) Unrelated cord blood transplantation in children with sickle cell disease: review of four-center experience. Pediatr Transplant 11(6): 641-644.

2. Arumugam PL, Scholes J, Perelman N, Xiao P, Yee JK, Malik P (2007) Improved human beta-globin expression from self-inactivating lentiviral vectors carrying the chicken hypersensitive site-4 (cHS4) insulator element. Mol Ther 15(10): 1863-1871.

3. Bank A, Dorazio R, Leboulch P (2005) A phase I/II clinical trial of beta-globin gene therapy for beta-thalassemia. Ann N Y Acad Sci 1054: 508-316.

4. Bell AC, West AG, Felsenfeld G (1999) The protein CTCF is required for the enhancer blocking activity of vertebrate insulators. Cell 98(3): 387-396.

5. Bell AC, West AG, Felsenfeld G (2001) Insulators and boundaries: versatile regulatory elements in the eukaryotic genome. Science 291(5503): 447-450.

6. Bender MA, Gelinas RE, Miller AD (1989) A majority of mice show long-term expression of a human beta-globin gene after retrovirus transfer into hematopoietic stem cells. Molecular and cellular biology 9(4): 1426-1434.

7. Beutel G, Meyer J, Ma L, Yin S, Eder M, von Neuhoff N, Wilkens L, Wei J, Hertenstein B, Heil G, Schlegelberger B, Ganser A, Li Z, Baum C (2005) Expression of the p75 neurotrophin receptor in acute leukaemia. Br J Haematol 131(1): 67-70.

8. Bibikova M, Carroll D, Segal DJ, Trautman JK, Smith J, Kim YG, Chandrasegaran S (2001) Stimulation of homologous recombination through targeted cleavage by chimeric nucleases. Molecular and cellular biology 21(1): 289-297.

9. Bodine DM, Karlsson S, Nienhuis AW (1989) Combination of interleukins 3 and 6 preserves stem cell function in culture and enhances retrovirus-mediated gene transfer into hematopoietic stem cells. Proceedings of the National Academy of Sciences of the United States of America 86(22): 8897-8901.

10. Boulad F, Giardina P, Gillio A, Kornan N, Small T, Brochstein J, Van Syckle K, George D, Szaboecs P, O'Reilly RJ (1998) Bone marrow transplantation for homozygous beta-thalassemia. The Memorial Sloan-Kettering Cancer Center experience. Ann N Y Acad Sci 850: 498-502.

11. Burguess-Beusse B, Farrell C, Gaszner M, Litt M, Mutskov V, Recillas-Targa F, Simpson M, West A, Felsenfeld G (2002) The insolation of genes from external enhancers and silencing chromatin. Proceedings of the National Academy of Sciences of America I: 1.

12. Bush S, Mandel FS, Giardina PJ (1998) Future orientation and life expectations of adolescents and young adults with thalassemia major. Ann N Y Acad Sci 850: 361-369.

13. Calmels B, Ferguson C, Laukkanen MO, Adler R, Faulhaber M, Kim HJ, Sellers S, Hematti P, Schmidt M, von Kalle C, Akagi K, Donahue RE, Dunbar CE (2005) Recurrent retroviral vector integration at the Mds1/Evi1 locus in nonhuman primate hematopoietic cells. Blood 106(7): 2530-2533.

14. Carey BW, Markoulaki S, Hanna J, Saha K, Gao Q, Mitalipova M, Jaenisch R (2009) Reprogramming of murine and human somatic cells using a single polycistronic vector. Proceedings of the National Academy of Sciences of the United States of America 106(1): 157-162.

15. Case SS, Price MA, Jordan CT, Yu XJ, Wang L, Bauer G, Haas DL, Xu D, Stripecce R, Naldini L, Kohn DB, Crooks GM (1999) Stable transduction of quiescent CD34+(+)CD38(-) human hematopoietic cells by HIV-1-based lentiviral vectors. Proceedings of the National Academy of Sciences of the United States of America 96(6): 2988-2993.

16. Chang JC, Liu D, Kan YW (1992) A 36-base-pair core sequence of locus control region enhances retrovirally transferred human beta-globin gene expression. Proceedings of the National Academy of Sciences of the United States of America 89(7): 3107-3110.
17. Chin JY, Kuan JY, Lonkar PS, Krause DS, Seidman MM, Peterson KR, Nielsen PE, Kole R, Glazer PM (2008) Correction of a splice-site mutation in the beta-globin gene stimulated by triplex-forming peptide nucleic acids. Proceedings of the National Academy of Sciences of the United States of America 105(36): 13514-13519.

18. Chui DH, Hardison R, Riener C, Miller W, Carver MF, Molchanova TP, Efremov GD, Huisman TH (1998) An electronic database of human hemoglobin variants on the World Wide Web. Blood 91(8): 2643-2644.

19. Ciavattini DM, Ryan TM, Farmer SC, Tomines TM (1995) Mouse model of human beta zero thalassemia: targeted deletion of the mouse beta maj- and beta min-globin genes in embryonic stem cells. Proceedings of the National Academy of Sciences of the United States of America 92(20): 9259-9263.

20. Coffin JM, Hughes SH, Varmus HE (1997) Retrovirus: Cold Spring Harbor Laboratory Press.

21. Cone RD, Weber-Benarous A, Baorto D, Mulligan RC (1987) Regulated expression of a complete human beta-globin gene encoded by a transmissible retrovirus vector. Molecular and cellular biology 7(2): 887-897.

22. Crone TM, Pegg AE (1993) A single amino acid change in human O6-alkylguanine-DNA alkyltransferase decreasing sensitivity to inactivation by O6-alkylguanine. Cancer Res 53(20): 4750-4753.

23. Cunningham MJ, Macklin EA, Neufeld EJ, Cohen AR (2004) Complications of beta-thalassemia major in North America. Blood 104(1): 34-39.

24. Dave UP, Agarwala K, Tripathi R, Cleveland SM, Thompson MA, Yi M, Stephens R, Downing JR, Jenkins NA, Copeland NG (2009) Murine leukemias with retroviral insertions at Lmo2 are predictive of the leukemias induced in SCID-X1 patients following retroviral gene therapy. PLoS Genet 5(5): e1000491.

25. Dominski Z, Kole R (1993) Restoration of correct splicing in thalassemia pre-mRNA by antisense oligonucleotides. Proc Natl Acad Sci U S A 90(18): 8673-8677.

26. Dzierzak EA, Papayannopoulou T, Mulligan RC (1988) Lineage-specific expression of a human beta-globin gene in murine bone marrow transplant recipients reconstituted with retrovirus-transduced stem cells. Nature 331(6151): 35-41.

27. Emery DW, Morrisey F, Li Q, Stamatoyannopoulos G (1999) Analysis of gamma-globin expression cassettes in retrovirus vectors. Hum Gene Ther 10(6): 877-888.

28. Emery DW, Yannaki E, Tubb J, Nishino T, Li Q, Stamatoyannopoulos G (2002) Development of virus vectors for gene therapy of beta chain hemoglobinopathies: flanking with a chromatin insulator reduces gamma-globin gene silencing in vivo. Blood 100(6): 2012-2019.

29. Emery DW, Yannaki E, Tubb J, Stamatoyannopoulos G (2000) A chromatin insulator protects retrovirus vectors from chromosomal position effects. Proceedings of the National Academy of Sciences of the United States of America 97(16): 9150-9155.

30. Evans-Galea MV, Wielgosz MM, Hanawa H, Srivastava DK, Nienhuis AW (2007) Suppression of clonal dominance in vivo avoids subsequent stem cell gene silencing and age-dependent complications of beta-thalassemia: an optimistic update. Semin Hematol 44(1): 5-14.

31. Fragkos M, Manougian NP, Tubb J, Emery DW (2005) Use of the hereditary persistence of fetal hemoglobin 2 enhancer to increase the expression of oncoretrovirus vectors for human gamma-globin. Gene therapy 12(21): 1591-1600.

32. Fu XH, Liu DP, Liang CC (2002) Chromatin structure and transcriptional regulation of the beta-globin locus. Experimental cell research 278(1): 1-11.

33. Fucharoen S, Winichagoon P (2000) Clinical and hematologic aspects of hemoglobin E-thalassemia. Curr Opin Hematol 7(2): 106-112.

34. Gardenghi S, Marongiu MF, Ramos P, Guy E, Breda L, Chadburn A, Liu Y, Amargiro N, Rechavi G, Rachmilewitz EA, Breuer W, Cabantchik ZI, Wrighting DM, Andrews NC, de Sousa M, Giordana PJ, Grady RW, Rivella S (2007) Ineffective erythropoiesis in {beta}-thalassemia is characterized by increased iron absorption mediated by down-regulation of hepcidin and up-regulation of ferroportin. Blood 109(11): 5027-5035.

35. Gerson SL (2000) Drug resistance gene transfer: Stem cell protection and therapeutic efficacy. Exp Hematol 28(12): 1315-1324.

36. Giardina PJ, Grady RW (2001) Chelation therapy in beta-thalassemia: an optimistic update. Semin Hematol 38(4): 360-366.

37. Giardine B, van Bael S, Kaimakis P, Riemer C, Miller W, Samara M, Kollia P, Anagnostou NP, Chui DH, Wajcenberg L, Hardison RC, Patinos GP (2007) HbVar database of human hemoglobin variants and thalassemia mutations: 2007 update. Hum Mutat 28(2): 206.

38. Giardina C, Lucareli G (1994) Bone marrow transplantation in the treatment of thalassemia. Curr Opin Hematol 1(2): 170-176.

39. Gorman L, Suter D, Emerick V, Schumperli D, Kole R (1998) Stable alteration of pre-mRNA splicing patterns by modified U7 small nuclear RNAs. Proc Natl Acad Sci U S A 95(9): 4929-4934.

40. Greaves DR, Fraser P, Vidal MA, Hedjes PM, Ropers D, Luzzatto L, Grosved F (1990) A transgenic mouse model of sickle cell disease [see comments]. Nature 343(6254): 183-185.

41. Han XD, Lin C, Chung J, Sadelain M, Kan YW (2007) Fetal gene therapy of alpha-thalassemia in a mouse model. Proceedings of the National Academy of Sciences of the United States of America 104(21): 9007-9011.

42. Hanawa H, Yamamoto M, Zhao H, Shimada T, Persons DA (2009) Optimized lentiviral vector design improves titer and transgene expression of vectors containing the chicken beta-globin locus H4S insulinator element. Mol Ther 17(4): 667-674.

43. Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Grosveld F, van Assendelft GB, Greaves DR, Kollia P (1987) Regulated expression of a complete human beta-globin gene encoded by a transmissible retrovirus vector. Molecular and cellular biology 7(2): 887-897.

44. Hansen G, Nikorev E, Introna J, Donohue T, Forni L, Grady RW, Rivella S (2007) Increased expression of genes in beta-thalassemic hematopoietic cells. Mol Ther 16(3): 525-533.

45. Hongeng S, Pakakasama S, Chuanumrit S, Sirachainan N, Sura T, Ungkanont A, Chuncharunee S, Jootar S, Issaragisil S (2002) Permanent and panerythrocytoid correction of murine beta-thalassemia: an optimistic update. Semin Hematol 39(1): 667-674.

46. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulfraat N, Leboulch P, Lim A, Osborne CS, Pawliuk R, Morillon E, Sorensen R, Forster A, Fraser P, Cohen JJ, de Saint Basile G, Allez M, Wernig M, Markoulaki S, Sun CW, Meissner A, Stoppa-Lyneton D, Romana S, Radford-Weiss I, Gross F, Valensi F, Delabesse E, Macintyre E, Sigaux F, Soulier J, Leiva LE, Wissler M, Prinz C, Rabbits TH, Le Deist F, Fischer A, Cavazzana-Calvo M (2003) LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 302(5648): 415-419.

47. Hunan H, Baweja AK, Mironov N, Li Q, Tubb J, Emery DW (2004) High-level beta-globin expression and preferred intragenic integration after lentiviral transduction of human cord blood stem cells. J Clin Invest 114(7): 953-962.

48. Imren S, Payen E, Westerman KA, Pawliuk R, Tang P, Rosten PM, Nagel RL, Leboulch P, Eaves CJ, Humphries RK (2004) High-level beta-globin expression and preferred intragenic integration after lentiviral transduction of human cord blood stem cells. J Clin Invest 114(7): 953-962.

49. Imren S, Payen E, Westerman KA, Pawliuk R, Fabry ME, Eaves CJ, Cavilla B, Wadsworth LD, Beuzard Y, Bouhassira EE, Russell R, London IM, Nagel RL, Leboulch P, Humphries RK (2002) Permanent and panerythroidic correction of murine beta thalassemia by multiple lentiviral integration in hematopoietic stem cells. Proceedings of the National Academy of Sciences of the United States of America 99(22): 14380-14385.

50. Kalberer CP, Pawliuk R, Imren S, Bachelet T, Takekoshi KJ, Fabry M, Eaves CJ, London IM, Humphries RK, Leboulch P (2000) Preselection of retrovirally transduced bone marrow avoids subsequent stem cell gene silencing and age-dependent
extinction of expression of human beta-globin in engrafted mice. PG - 5411-5. Proceedings of the National Academy of Sciences of the United States of America 97(10): 5411-5415.

51. Karlsson S, Bodine DM, Perry L, Papayannopoulou T, Nienhuis AW (1988) Expression of the human beta-globin gene following retroviral-mediated transfer into multipotential hematopoietic progenitors of mice. Proceedings of the National Academy of Sciences of the United States of America 85(16): 6062-6066.

52. Karlsson S, Papayannopoulou T, Schweiger SG, Stamatoyannopoulos G, Nienhuis AW (1987) Retroviral-mediated transfer of genomic globin genes leads to regulated production of RNA and protein. Proceedings of the National Academy of Sciences of the United States of America 84(8): 2411-2415.

53. Kim YJ, Kim YS, Larochelle A, Renaud G, Wolfeberg TG, Adler R, Donahue RE, Hematti P, Hong BK, Roayaei J, Akagi K, Riberdy JM, Nienhuis AW, Dunbar CE, Persons DA (2009) Sustained high-level polyclonal hematopoietic marking and transgene expression 4 years after autologous transplantation of rhesus macaques with SIV lentiviral vector-transduced CD34+ cells. Blood 113(22): 5434-5443.

54. La Nasa G, Argioli F, Giardini C, Pession A, Fagioli F, Capecchi M, Vacca A, De Stefano P, Pirrodi A, Littera G, Nesci S, Locatelli F (2005) Unrelated bone marrow transplantation for beta-thalassemia patients: The experience of the Italian Bone Marrow Transplant Group. Ann N Y Acad Sci 1054: 186-195.

55. Lacerra G, Sierakowska H, Carestia C, Bucharenov S, Summerton J, Weller D, Kole R (2000) Restoration of hemoglobin A synthesis in erythroid cells from peripheral blood of thalassemia patients. Proceedings of the National Academy of Sciences of the United States of America 97(17): 9591-9596.

56. Leboulch P, Huang GM, Humphries RK, Oh YH, Eaves CJ, Tuan DY, London DM (1994) 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 13(13): 3065-3076.

57. Levasseur DN, Ryan TM, Pavlik KM, Townes TM (2003) Correction of a mouse model of sickle cell disease: lentiviral/antisickling beta-globin gene transduction of unmobilized, purified hematopoietic stem cells. Blood 102(13): 4312-4319.

58. Levasseur DN, Ryan TM, Reilly MP, McCune SL, Asakura T, Holmes MC, Leboulch P (2007) Successful correction of the human beta-thalassemia major phenotype using a lentiviral vector flanked by the chicken hypersensitive site 4 chromatin insulator. Blood 109(7): 2388-2429.

59. Maquat LE (2005) Nonsense-mediated mRNA decay in mammals. J Cell Sci 118(Pt 9): 1773-1776.

60. May C, Rivella S, Callegari J, Heller G, Gaensler KM, Luzzatto L, Sadelain M (2000) Therapeutic haemoglobin synthesis in beta-thalassaemic mice expressing lentivirus-encoded human beta-globin. Nature 406(6791): 82-86.

61. May C, Rivella S, Chadbourn A, Sadelain M (2002) Successful treatment of murine beta-thalassaemia intermedia by transfer of the human beta-globin gene. Blood 99(6): 1902-1908.

62. Mcioce A, Cesari R, Lotti F, Rossi C, Sanvito F, Ponzi M, Routledge SJ, Chow CM, Antoniou MN, Ferrari G (2008) In vivo selection of genetically modified erythropoietic progenitors leads to long-term correction of beta-thalassemia. Proceedings of the National Academy of Sciences of the United States of America 105(30): 10547-10552.

63. Miller JL, Walsh CE, Nay PA, Samulski RJ, Nienhuis AW (1993) Single-copy transduction and expression of human gamma-globin in K562 erythroleukemia cells using recombinant adenovirus-associated virus vectors: the effect of mutations in NF-E2 and GATA-1 binding motifs within the hypersensitivity site 2 enhancer [published erratum appears in Blood 1995 Feb 1;85(3):362]. Blood 82(6): 1900-1906.

64. Milner PF, Clegg JB, Weatherall DJ (1971) Haemoglobin-H disease due to a unique haemoglobin variant with an elongated alpha-chain. Lancet 1(7702): 729-732.

65. Murari J, Smith LL, Wilson JB, Schneider RG, Huisman TH (1977) Some properties of hemoglobin Gun Hill. Hemoglobin 1(3): 267-282.

66. Nishino T, Tubb J, Emery DW (2006) Partial correction of murine beta-thalassemia with a gammaretrovirus vector for human gamma-globin. Blood Cells Mol Dis 37(1): 1-7.

67. Novak U, Harris EA, Forrester W, Groudone M, Gelinhas R (1990) High-level beta-globin expression after retroviral transfer of locus activation region-containing human beta-globin gene derivatives into murine erythroleukemia cells. Proceedings of the National Academy of Sciences of the United States of America 87(9): 3386-3390.

68. Pab CO, Peisach E, Grant RA (2001) Design and selection of novel Cys2His2 zinc finger proteins. Annual review of biochemistry 70: 313-340.

69. Pavlik R, Westerman KA, Fabry ME, Payen E, Tighe R, Bouhaissia EE, Acharaya SA, Ellis J, London DM, Eaves CJ, Humphries RK, Beuzard Y, Nagel RL, Leboulch P (2001) Correction of sickle cell disease in transgenic mouse models by gene therapy. Science 294(5550): 2368-2371.

70. Pike-Overzet K, van der Burg M, Wagemaker G, van Dongen JJ, Staal FJ (2007) New insights and unresolved issues regarding insertional mutagenesis in X-linked SCID gene therapy. Mol Ther 15(1): 1019-1916.

71. Plavec I, Papayannopoulou T, Maury C, Meyer F (1993) A human beta-globin gene fused to the human beta-globin locus control region is expressed at high levels in erythroid cells of mice transfected with retrovirus-transduced hematopoietic stem cells. Blood 81(5): 1384-1392.

72. Porteus MH, Carroll D (2005) Gene targeting using zinc finger nucleases. Nature biotechnology 23(8): 967-973.

73. Puthenveetil G, Scholes J, Carbonell D, Qureshi N, Xia P, Zeng L, Li S, Yu Y, Hits AL, Yee JK, Malik P (2004) Successful correction of the human beta-thalassaemia major phenotype using a lentiviral vector. Blood 104(12): 3445-3453.

74. Raffopoulos H, Ward M, Leboulch P, Bank A (1997) Long-term transfer and expression of the human beta-globin gene in a mouse transplant model. Blood 90(9): 3414-3422.
Sazani P, Kole R (2003) Therapeutic potential of antisense ... Wilsons (DA) (2000) Direct reversal of DNA damage by mutant methlytransferase protein protects mice against dose-intensified chemotheraphy and leads to in vivo selection of hematopoetic stem cells. Cancer Res 60(18): 5187-5195.

Rechavi G, Rivella S (2008) Regulation of iron absorption in hemoglobinopathies. Curr Mol Med 8(7): 646-662.

Ren S, Wong BY, Li J, Luo XN, Wong PM, Atweh GF (1996) Production of genetically stable high-titer retroviral vectors that carry a human gamma-globin gene under the control of the alpha-globin locus control region. Blood 87(6): 2518-2524.

Rivella S, Callegari JA, May C, Tan CW, Sadelain M (2000) The CHS4 insulator increases the probability of retroviral expression at random chromosomal integration sites. J Virol 74(10): 4679-4687.

Rivella S, May C, Chadburn A, Riviere I, Sadelain M (2003) A novel murine model of Cooley anemia and its rescue by lentiviral-mediated human beta-globin gene transfer. Blood 101(8): 2932-2939.

Rogers FA, Vasquez KM, Egholm M, Glazer PM (2002) Site-directed recombination via bifunctional PNA-DNA conjugates. Proceedings of the National Academy of Sciences of the United States of America 99(26): 16695-16700.

Sabatino DE, Seidel NE, Aviles-Mendoza GJ, Cline AP, Anderson SM, Gallagher PG, Bodine DM (2000a) Long-term expression of gamma-globin mRNA in mouse erythrocytes from retrovirus vectors containing the human gamma-globin gene fused to the ankyrin-1 promoter. Proceedings of the National Academy of Sciences of the United States of America 97(24): 13294-13299.

Sabatino DE, Wong C, Cline AP, Pyle L, Garrett LJ, Gallagher PG, Bodine DM (2000b) A minimal ankyrin promoter linked to a human gamma-globin gene demonstrates erythroid specific copy number dependent expression with minimal position or enhancer dependence in transgenic mice. J Biol Chem 275(37): 28549-28554.

Sadelain M, Boulad F, Galanello R, Giardina P, Locatelli F, Sabatino DE, Wong C, Cline AP, Rogers FA, Vasquez KM, Egholm M, Glazer PM (2002) Site-directed repair of thalassemic human beta-globin mRNA in vivo using antisense oligonucleotides as modulators of alternative splicing. J Clin Invest 110(8): 879-887.

Sadelain M, Boulad F, Galanello R, Giardina P, Locatelli F, Sabatino DE, Wong C, Cline AP, Rogers FA, Vasquez KM, Egholm M, Glazer PM (2002) Site-directed repair of thalassemic human beta-globin mRNA in vivo using antisense oligonucleotides. Mol Pharmacol 62(3): 545-553.

Sadelain M (2006) A genetic strategy to treat sickle cell anemia by coregulating globin transgene expression and RNA interference. Nature biotechnology 24(1): 89-94.

Sazani P, Kole R (2003) Therapeutic potential of antisense oligonucleotides as modulators of alternative splicing. J Clin Invest 112(4): 481-486.

Schambach A, Baum C (2008) Clinical application of lentiviral vectors - concepts and practice. Curr Gene Ther 8(6): 474-482.

Sierakowska H, Sambade MJ, Agrawal S, Kole R (1996) Repair of thalassemic human beta-globin mRNA in mammalian cells by antisense oligonucleotides. Proceedings of the National Academy of Sciences of the United States of America 93(23): 12840-12844.