Available online at www.sciencedirect.com

ScienceDirect

Biomedical Journal

journal homepage: www.elsevier.com/locate/bj

Review Article

Current and future alternative therapies for beta-thalassemia major

Edouard de Dreuzy [a,b,1], *Kanit Bhukhai* [a,b,1], *Philippe Leboulch* [a,b,c,d], *Emmanuel Payen* [a,b,e,*]

[a] CEA, Institute of Emerging Diseases and Innovative Therapies, Fontenay aux Roses, France
[b] University of Paris 11, CEA-iMETI, 92260 Fontenay aux Roses, France
[c] Department of Medicine, Harvard Medical School and Genetics Division, Brigham and Women's Hospital, Boston MA, USA
[d] Mahidol University and Ramathibodi Hospital, Bangkok, Thailand
[e] INSERM, Paris, France

Dr. Emmanuel Payen

ARTICLE INFO

Article history:
Received 11 March 2015
Accepted 12 October 2015
Available online 6 April 2016

Keywords:
Beta-thalassemia
Allogeneic transplantation
Beta-globin
Gene therapy
Gamma-globin inducers

ABSTRACT

Beta-thalassemia is a group of frequent genetic disorders resulting in the synthesis of little or no β-globin chains. Novel approaches are being developed to correct the resulting α/β-globin chain imbalance, in an effort to move beyond the palliative management of this disease and the complications of its treatment (e.g. life-long red blood cell transfusion, iron chelation, splenectomy), which impose high costs on healthcare systems. Three approaches are envisaged: fetal globin gene reactivation by pharmacological compounds injected into patients throughout their lives, allogeneic hematopoietic stem cell transplantation (HSCT), and gene therapy. HSCT is currently the only treatment shown to provide an effective, definitive cure for β-thalassemia. However, this procedure remains risky and histocompatible donors are identified for only a small fraction of patients. New pharmacological compounds are being tested, but none has yet made it into common clinical practice for the treatment of beta-thalassemia major. Gene therapy is in the experimental phase. It is emerging as a powerful approach without the immunological complications of HSCT, but with other possible drawbacks. Rapid progress is being made in this field, and long-term efficacy and safety studies are underway.

Beta-thalassemia was first discovered in the Mediterranean Basin and is highly prevalent in countries also affected by malaria, but human migration has resulted in the establishment of this disease in many areas of the world [1]. All patients display defects of hemoglobin (Hb) beta-chain production, but the resulting phenotypes are highly variable, ranging from severe anemia to an absence of clinical symptoms. Classification and severity grading are based principally on spontaneous Hb levels and clinical tolerance, regardless of the underlying genotype. Patients with beta-

* *Corresponding author*. CEA, Institute of Emerging Diseases and Innovative Therapies, 18, Route du Panorama, BP6, 92265 Fontenay-aux Roses Cedex, France. Tel.: +33 1 46547055; fax: +33 1 46547499.
E-mail address: emmanuel.payen@cea.fr (E. Payen).

Peer review under responsibility of Chang Gung University.

[1] These authors contributed equally to the writing of this review.

http://dx.doi.org/10.1016/j.bj.2015.10.001

thalassemia intermedia have blood Hb concentrations of 7–10 g/dL and do not require regular transfusion. They may display a broad spectrum of clinical signs, depending on the degree of alpha to non-alpha globin chain imbalance and several genetic and environmental factors. They may suffer from numerous complications, including pulmonary hypertension, thrombotic events, infection, endocrine dysfunction and leg ulcers [2]. Patients with beta-thalassemia major require regular transfusions of red blood cells to survive [3]. However, repeated transfusions cause iron overload, with life-threatening complications, such as endocrine dysfunction, cardiomyopathy, liver disease and, ultimately, premature death. In the absence of transfusion, patients with beta-thalassemia major die within the first five years of life, and even with transfusions, only 50–65% of patients live beyond the age of 35 years in high-income countries [4–6]. Research is highly active [Fig. 1], and progress has been made towards the development of new drugs, including biological products, some of which have recently reached the clinical trial stage.

Pharmacological therapies

Trace amounts of fetal Hb (HbF) persist into adulthood, accounting for less than 1% of total Hb in most adults [7]. However, HbF levels may exceed this threshold in some individuals. Beta-thalassemia patients with inherited persistent high levels of HbF production have a milder clinical course than other patients with this disease, and many do not require transfusions [8]. Therapeutic approaches reactivating HbF, and increasing its concentration, are, therefore, attractive. Variant HbF levels are highly inheritable [9]. Genome-wide association studies (GWAS) have compared individuals with low and persistently high levels of HbF, with the aim of identifying quantitative trait loci (QTL) to serve as a source of plausible candidate causal genes or regulatory regions [10] accounting for the persistence of γ-globin gene expression. Strong associations between HbF level and single-nucleotide polymorphisms (SNPs) have been identified for at least four genomic loci, including the HBB (hemoglobin, beta) and olfactory receptor gene clusters on chromosome 11p15.4, the chromosome 6q23.3 HBS1L-MYB (HBS1-like translational GTPase - v-myb avian myeloblastosis viral oncogene homolog) intergenic region (HMIP), the BCL11A (B-cell CLL/lymphoma 11A) locus on chromosome 2p16.1, and the KlF1 (Kruppel-like factor 1) gene on chromosome 19p13.13 [11–18]. Variants at the HMIP-BCL11A-HBB loci account for 20–50% of the variability of HbF levels and the relative contributions of these variants differ between ethnic groups [19–23].

The −158C > T (rs7482144) SNP located at the *Xmn*I site of the HBG2 (hemoglobin gamma G gene) promoter in the HBB locus was shown to be correlated with HbF levels in pioneering studies conducted on normal individuals and patients with sickle cell disease or β-thalassemia [24–27]. In the absence of a demonstrated functional role for this site, it has been suggested that HbF phenotype is modified by *cis*-linked elements located elsewhere in the β-globin cluster and in linkage disequilibrium with this SNP [28,29]. Indeed, a quasi-palindromic structure located at the 5′ DNAse hypersensitive site 4 (HS4) of the locus control region (LCR), a polymorphism of which is in linkage disequilibrium with the *Xmn*I site [28], may affect direct or indirect interactions with the transcriptional repressor BCL11A [30]. This transcriptional repressor directly regulates HbF levels during the globin switch after birth [31]. Erythroid-specific BCL11A knockdown blocks the silencing of fetal globin genes [32], and BCL11A SNPs associated with HbF level variations (located in intron 2) colocalize with target sites for erythroid transcription factors [33]. The KLF1 transcription factor represses γ-globin expression by activating BCL11A, with haploinsufficiency causing high HbF levels [17,34,35]. The MYB gene is a key regulator of the balance between proliferation and differentiation during erythropoiesis [36]. It regulates HbF levels through an as yet undetermined mechanism [37,38]. The intergenic HBS1L-MYB region contains MYB enhancer sites and DNA targets for erythroid transcription factors [39]. Genetic variants associated with the persistence of HbF, located in a 24 kb region of HMIP [15], affect MYB expression [16] by reducing erythroid transcription factor binding and long-range promoter activation [40].

The regulatory transcription factors involved in γ-globin gene regulation or F-cell differentiation and survival are potentially of considerable interest as targets for increasing HbF levels. However, it remains difficult to modulate the function of factors other than enzymes or signal-dependent nuclear factors by disrupting DNA/protein or protein/protein interactions [41] and such modulation is particularly problematic for factors with important non-erythroid functions [42]. Furthermore, any interference with erythroid transcription factors may result in the inappropriate disruption of erythropoiesis. Efforts are being made to design endonucleases capable of precisely disrupting the genomic sequences involved in the erythroid-specific expression of γ-globin repressors, as a means of activating HbF, but this remains a difficult challenge [43].

The S-phase cell-cycle inhibitor hydroxyurea (HU) has proved clinically effective in patients with sickle cell anemia (SCA) [44,45]. It is also of clinical benefit to some patients with β-thalassemia intermedia and it reduces the need for transfusions in a subset of individuals with β-thalassemia major [46–53]. However, side effects have been reported, including cytopenia, hyperpigmentation, weight gain, opportunistic infections, azoospermia in approximately 80% of men (even years after the end of treatment), and marked hypomagnesemia [54]. There is little or no risk of leukemia [55], but HU is believed to be teratogenic [54]. Given the potential adverse effects and reported efficacy in only a subset of patients, it is important to identify likely responders and nonresponders before initiating treatment, to ensure that prescriptions are efficiently targeted. The increase in HbF levels following HU injection in patients with β-thalassemia major seems to be correlated with the *Xmn*I polymorphism [46,49,50,53], although this result has not been confirmed by all studies [47]. The correlation is less evident for non-transfusion-dependent thalassemia [48,51,52]. This is problematic, as treatments aiming to increase Hb levels by a few grams per deciliter are clearly more promising for the treatment of patients with thalassemia intermedia than for the treatment of transfusion-dependent patients. Baseline HbF level is clearly correlated with BCL11A SNP markers [11,12,14] and levels of the γ-globin repressor

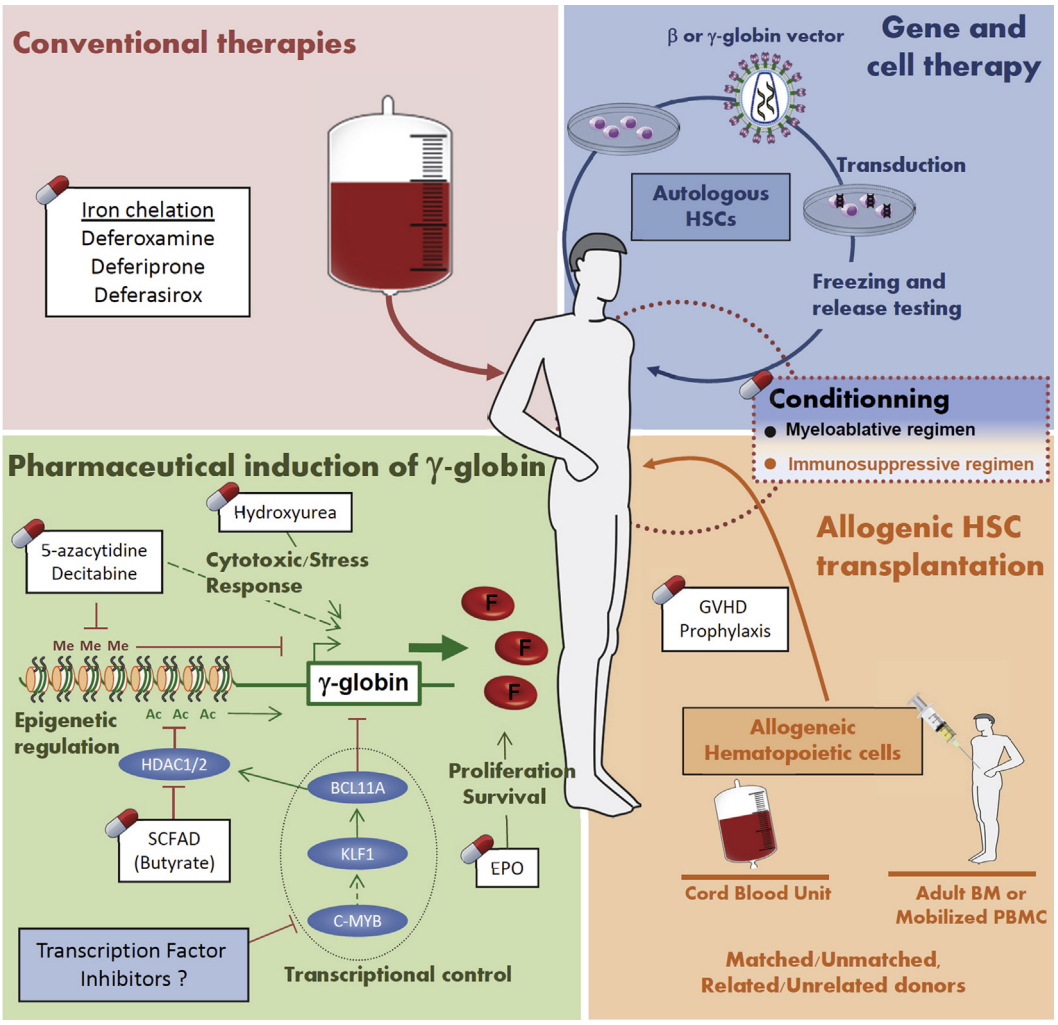

Fig. 1 – Current and future therapies for beta-thalassemia major. Hemoglobin disorders account for almost 5% of deaths in children under the age of five years. For a minority of patients, mostly in high-income countries, current therapies include life-long monthly supportive red blood cell transfusions together with iron chelation or curative allogeneic HSC transplantation. The pharmacological induction of fetal hemoglobin and gene therapy are currently at the experimental stage. Top left: Conventional therapy includes regular red blood cell transfusions and iron chelation with injectable (Deferoxamine) or oral (Deferiprone or Deferasirox) drugs. Bottom left: Gamma-globin chain inducers aim to reduce the need for red blood cell transfusions. A number of drugs have been tested, including cytotoxic compounds and epigenetic regulators. The first drug shown to increase γ-globin expression was the demethylating agent 5-azacitidine. Small-chain fatty acid derivatives, including arginine butyrate, have also been shown to increase γ-globin expression most likely by inhibiting histone deacetylation. Hydroxyurea is the only drug currently approved for γ-globin induction. It acts through multiple mechanisms. Its cytotoxic activity is thought to accelerate the differentiation process and to stimulate cellular stress response pathways, leading to an overall increase in the number of F cells. Gamma-globin gene induction by other cytotoxic agents may also be mediated by this stress response. Erythropoietin (EPO) has proliferative and anti-apoptotic properties. The combined administration of recombinant EPO together with cytotoxic drugs can be beneficial for patients with low baseline EPO levels. Future treatments may target the transcription factors involved in γ-globin repression, such as BCL11a and KLF1. BCL11a is an essential transcription factor involved in γ-globin downregulation. It binds to intergenic regions of the HBB locus, promoting long-range interactions with the LCR that favors β-globin expression. It recruits histone deacetylase to repress γ-globin. KLF1 is a strong inducer of β-globin expression that also activates BCL11A transcription when produced in large amounts in adult cells. KLF1 may itself be stimulated by c-Myb. Bottom right: Allogeneic transplantation with related or unrelated donor cells from cord blood units, mobilized peripheral blood or bone marrow is currently the only curative treatment, provided that a compatible donor can be found. Yet the risks of graft versus host disease and transplant rejection restrict the use of this procedure. Top right: Gene therapy is a promising single-dose medicine, without the need for immunosuppressive conditioning and graft versus host disease prophylaxis. It is potentially applicable to all patients. Studies evaluating safety and efficacy are currently underway and have already reported encouraging results. Studies of larger numbers of patients are required before any firm conclusions about efficacy and safety can be drawn. Long-term effectiveness/risk/cost ratios will need to be carefully addressed.

BCL11A [56], in both normal individuals and patients with beta-hemoglobinopathies [57]. However, only a few studies have investigated the correlation between polymorphisms and the HbF response to HU treatment, and conflicting results have been obtained [53,58–60]. In a study of beta-thalassemia patients of Iranian origin, polymorphisms of intron 2 of *BCL11A* were found to be correlated with the response to HU treatment [53]. By contrast, in studies including patients with SCA, BCL11A levels and SNPs were not found to be correlated with HbF induction after HU treatment [58–60], suggesting an important role for BCL11A in controlling baseline HbF levels, but with other factors required for HU to increase HbF levels. It has also been shown that intergenic HBS1L-MYB SNPs are not correlated with HU treatment [53,60].

SNPs of HBG2, HBS1L-MYB and BCL11A can be used for the rapid screening of disease severity in newborns and to provide more personalized care [61], but pharmacogenomics approaches do not distinguish between patients responding well and poorly to HU treatment [62]. The criteria for treatment should therefore not be based on these genetic characteristics. A number of genes involved in translation, ribosomal assembly and chromosome organization, but with no known function in normal, physiological β-globin switching, are deregulated in the erythroid cells of SCA patients treated with HU [58]. As recently suggested, the difference between weak and strong responses to HU may lie in the constitutive activation of genes involved in the stress response and terminal erythroid differentiation, and of genes protecting cells against stress-induced apoptosis in patients with strong responses [63]. Thus, the culture of erythroid progenitors and investigations of the expression profile of specific genes may make it easier to distinguish responders from nonresponders before the initiation of HU treatment in beta-thalassemia patients, but this possibility requires further study. Short trial periods on HU therapy (<3 months) are currently required to identify the patients most likely to respond to this treatment [48].

A number of other non-selective compounds, including cytotoxic compounds, short-chain fatty acid derivatives (SCFAD) and erythropoietin (Epo), have been investigated and evaluated in clinical trials [64]. Some are thought to act via epigenetic mechanisms. This is not surprising given (i) the strong correlation between epigenetic modification and the developmental pattern of globin gene expression [65–67] and (ii) the finding that some of the variation of HbF synthesis in adults may be linked to loci sensitive to epigenetic regulation [68]. However, the mechanisms of action of these compounds are not fully understood because they belong to several categories, including cytotoxic and hypomethylating agents (5-azacytidine, decitabine, HU), cytotoxic and histone deacetylase inhibitors, such as butyrate derivatives, and anti-apoptotic factors (Epo or Kit ligand) [69].

Allogeneic hematopoietic stem cell transplantation

Conventional therapies (red blood cell transfusion and iron chelation) improve the quality of life and survival of patients [70], but allogeneic hematopoietic stem cell transplantation (HSCT) currently offers the only hope of a definitive cure for patients with beta-thalassemia. HSCT was first successfully performed more than 30 years ago [71,72]. Several transplantation centers have since tried to categorize risk factors and to diversify HSCT procedures, with the aim of treating more patients, decreasing the risks of morbidity and mortality, and improving overall thalassemia-free survival. Several factors have been shown to affect patient outcome: severity criteria before transplantation (hepatomegaly, portal fibrosis and irregular chelation history), age at transplantation, stem cell source (peripheral blood, bone marrow, cord blood), histocompatibility (related matched, unrelated matched, mismatched, haploidentical), preparative conditioning regimen and pretransplant eradication of marrow hyperplasia. Despite the lack of evidence from randomized clinical studies comparing donor sources and/or conditioning regimens, HSCT is now widely accepted as a curative treatment [73] resulting in a long-term quality of life similar to that of the general population and higher than that of conventionally treated patients without active disease [74,75].

In a pioneering study, the group of Pesaro [76,77] assigned young patients to three classes according to the absence or presence of one, two or three risk factors before transplantation: hepatomegaly > 2 cm, portal fibrosis and irregular chelation history (class 1 = no risk factor, class 2 = one or two risk factors and class 3 = three risk factors). The transplantation of HLA (human leukocyte antigen)-matched sibling donor (MSD) cells into young class 1 or class 2 individuals on myeloablative conditioning (14 mg/kg busulfan + 200 mg/kg cyclophosphamide) and cyclosporin for graft-versus-host disease (GVDH) prophylaxis, gave excellent results (82–90% disease-free survival), but this treatment was highly toxic in pediatric class 3 patients (only 55% survived) [78,79]. Overall survival (OS) was increased to 74% by decreasing the dose of cyclophosphamide to below 200 mg/kg, but the probability of graft rejection (GR) remained high (35%) and event-free survival (EFS) remained low (49%) [80], probably due to inadequate immunosuppression and/or a failure to eradicate the massive erythroid hyperplasia.

Due to long-term exposure to iron overload, most patients over the age of 16 years have class 3 characteristics, and early attempts to treat them by transplantation, with high-dose cyclophosphamide regimens, were highly disappointing. All six patients died from graft failure or graft-versus-host disease (GVDH) in one study [81]. Use of a preparative regimen adapted to the risk factors identified in young patients improved the outcome for adult individuals, but OS remained low (63–65%). Rejection-free survival was slightly better than that for young class 3 patients (60–62%), mostly because rejection rates were lower in adults than in children, for unknown reasons [80–82]. Overall, the results obtained for class 3 patients were much worse than those for patients with class 1 or class 2 beta-thalassemia. Chronic active hepatitis at the time of transplantation had a strong negative impact on OS in adults [82].

Very few changes to the treatment of class 1 and 2 pediatric patients have occurred in recent years, with the exception of thiotepa, which is used to reduce rejection rates in patients under the age of four years [83]. A subgroup of young class III patients, those under the age of eight years, with

hepatomegaly >5 cm (class III high-risk (HR)) has been shown to have a much poorer clinical outcome after HSCT with a conventional conditioning regimen than other class III patients [84,85]. This subgroup of patients has a very high risk of GR and regimen-related toxicity. Lower intensity conditioning is thus required for both these patients and for adults [86]. Conflicting results for transplant-related mortality were obtained when the myeloablative regimen was changed from busulfan and cyclophosphamide to treosulfan, fludarabine and thiotepa (TreoFluT), and this change did not improve GVHD and graft failure in mixed-class groups of patients [87,88]. However, in well identified groups of class III patients and, even more significantly, in the subgroup of class III HR patients, the use of a TreoFluT regimen was associated with significant improvements in survival and EFS, due to a significant decrease in the incidence of sinusoidal obstruction syndrome (SOS) [89]. In this study, the increase in the risk of rejection due to the lower intensity of conditioning was counteracted by the use of peripheral blood stem cells (PBSC) rather than BM cells. In pediatric class 3 patients, ablation of the expanded thalassemic marrow and stronger immunosuppression through the use of an intensified preparation regimen including HU, azathioprine, fludarabine and hypertransfusion increased event-free survival to 80–85% [90,91]. The use of this protocol in adults did not decrease transplant-related mortality, and thalassemia-free survival remained low [92]. The inclusion of anti-thymocyte globulin (ATG) in the preparative regimen of pediatric patients from all classes, together with the adaptation of busulfan and cyclophosphamide concentrations, decreased the incidence of GVHD, minimized GR and transplant-related mortality and seemed to abolish the difference between patients from different Pesaro risk classes, in a recent survey carried out in Greece and France [93,94]. The transplantation of cord blood (CB) and bone marrow cells from a MSD offers a similar probability of long-term cure, but with a lower incidence of GVHD. The transplantation of PBSC yields lower rates of GR than bone marrow transplantation in class III patients receiving TreoFluT, but GVHD remains a concern when PBSC grafts are used [95].

One of the main barriers to HSCT with cells from HLA-identical siblings is the limited availability of donors, which is, theoretically, 25% for any single sibling. The combined use of pre-implantation genetic diagnosis and HLA matching provides a reliable source of stem cells from healthy siblings, but rates of successful transplantation after ovarian stimulation remain low [96]. For most of the remaining patients, millions of HLA types are stored in national registries. The probability of finding an unrelated donor (UD) with 9/10 or 10/10 HLA-matches (HLA-A, -B, -C, –DRB1 and -DQB1) on the basis of high-resolution typing exceeds 70% for patients of European ancestry [96], but this likelihood depends on ethnicity [97]. A prospective study of patients with hematologic malignancies suggested that the transplantation of cells with this degree of matching from an UD provides a likelihood of cure similar to that for the transplantation of cells from a MSD [98]. In both adults and children with β-thalassemia, bone marrow transplantation from an UD selected on the basis of high-resolution typing yields success rates similar to those obtained with a MSD [99,100], but with more severe GVHD. This approach is not widely used in adults, due to the high incidence of GVHD, transplant-related mortality and GR, but it may be used to treat children with class I/II β-thalassemia safely, provided that ATG is given [100]. A highly promising recently developed protocol including marrow reduction and pretransplant immunosuppression, followed by low-intensity conditioning with ATG, has been shown to result in a low risk of toxicity and durable engraftment in class 3 HR patients receiving cells from unrelated and related donors; this approach merits more widespread testing, even in individuals with lower risk β-thalassemia [101,102].

Donor cells can also be obtained from CB samples. The transplantation of CB cells originating from HLA-identical siblings is a viable alternative to the use of adult samples in class I/II patients [103–105]. Moreover, these cells have the theoretical advantages of tolerating a higher degree of HLA incompatibility than adult cells [106] and causing a lower incidence of acute and chronic GVHD [107]. However, in a report compiling data from 32 transplant centers from 1996 to 2009, survival and disease-free survival were as low as 62% and 20%, respectively, in patients with β-thalassemia receiving CB cells from mismatched donors, after myeloablative conditioning [108]. Graft failure was the chief cause of treatment failure other than death, which was linked to disease severity and the use of a non-adapted conditioning regimen. Multivariate analysis indicated that the principal factor associated with engraftment was the number of cells used. These results contrast with those of a Taiwanese study reporting the survival of 88% of young patients receiving one or two unrelated CB units after a full myeloablative regimen; 74% of these patients were disease-free five years after transplantation. Both chronic and acute GVHD occurred, but most cases were moderate [109]. However, double cord transplants were performed in this study if a single unit contained too few nucleated $CD34^+$ cells. More clinical trials are required.

Gene therapy

The concept of gene therapy for beta-thalassemia emerged a long time ago and was included in a plan for the viable treatment for hemoglobinopathies as early as 1978 at the University of California at Los Angeles (UCLA) [110]. However, major technical issues were encountered and attempts to demonstrate the efficacy of infusions of transfected mouse cells in myeloablated animals were far from convincing [111,112]. The regulatory globin sequences required for high levels of production [113] and efficient methods for gene introduction [114] were not available at the time. The stemness properties of the manipulated bone marrow cells were unknown. Early attempts to treat beta-thalassemia patients by inserting the β-globin gene into bone marrow cells, in 1980, were completely unsuccessful and received an avalanche of criticism [115,116].

It is essential to achieve a satisfactory combination of high levels of sustained β-globin expression and the stable propagation of complex sequences. More than 25 years were required to achieve these goals, which were made possible by

the characterization, isolation, size reduction, and blending of the β-globin locus regulatory elements and the advent of lentiviral vectors, which can transfer complex sequences into hematopoietic stem cells. The genetic elements required for the adult- and erythroid-specific expression of the β-globin gene were mapped close to the gene [117,118], in the promoter region, the second intron and downstream from the polyadenylation signal [119–121]. However, the combination of these elements in a single transgene yielded very low levels of β-globin gene expression in the erythroid cells of mice undergoing transplantation with genetically modified HSCs bearing the transgene [122–124]. The discovery of the β-globin LCR, located in a 15,000-base region far upstream from the globin genes [125–127], was a major step forward. The use of this region made it possible to design β-globin transgenes that were strongly expressed in the erythroid cells of transgenic mice [128,129]. Over a period of 10 years, efforts were made to delineate the enhancer core elements of the LCR [129,130], with the aim of decreasing the size of the DNA fragments used, such that the chimeric DNA constructs would be compatible with transfer vectors derived from gamma-retroviruses [131,132]. These vectors provided reasonable levels of β-globin transgene expression *in vitro*, but the transduction of mouse hematopoietic stem cells (HSCs) was suboptimal, resulting in limited and variable expression of the β-globin gene *in vivo* [133,134].

Gamma-retroviral vectors (γ-RVs) have three major defects: (i) limited cargo capacity, (ii) instability, and (iii) an inability to transduce non-dividing cells (most HSCs are quiescent [135]). These drawbacks precluded the efficient correction of beta-thalassemia by the infusion of genetically modified HSCs. As a result, the gene therapy community working on globin disorders rapidly abandoned the use of γ-RVs shortly after lentiviral vectors (LVs) derived from human immunodeficiency virus type 1 (HIV1) became available. Not only were LVs able to transduce cells arrested at the G1-S boundary of the cell cycle [136,137], they were also able to transfer much longer sequences than γ-RVs [138]. This key characteristic is dependent on a molecular mechanism for inhibiting the splicing of the viral RNA before its packaging into lentiviral particles. This mechanism involves active transport of the full-length (unspliced) viral RNA from the nucleus to the cytoplasm by the viral protein REV, which binds to the REV-responsive element (RRE) located within the viral RNA structure [139]. The potential utility of incorporating this mechanism into complex LCR/beta-globin LVs was suggested by our discovery that unwanted RNA splicing is the key determinant of LCR/beta-globin γ-RV instability and low titers, and that this problem could be largely corrected by the removal of undesirable splice sites [131]. Our groups and that of Sadelain then focused on obtaining proof-of-principle for the preclinical efficacy of LCR/beta-globin LVs in two mouse models of beta-hemoglobinopathies: murine beta-thalassemia [140] and transgenic mouse models of sickle cell disease [141]. This approach was subsequently consolidated by other studies [142–146]. For gene therapy for sickle cell disease, we decided to abandon the wild-type beta-globin gene, choosing instead to work with a variant we constructed, with an enhanced ability to inhibit HbS polymerization [141]. This variant (β^{A-T87Q}) was generated by introducing the codon for a specific amino-acid residue present in both the gamma- and delta-globin proteins (Q, glutamine) to replace the corresponding codon in beta-globin (T, Threonine) [147]. The β^{A-T87Q} variant has an additional key advantage for gene therapy trials in beta-thalassemia: it facilitates accurate quantification of the amount of vector-derived beta-globin produced or present in circulating red blood cells by high-performance liquid chromatography (HPLC), even in beta-thalassemia patients receiving red blood cell transfusions or with β^{+}-thalassemia [148]. Such quantification is not possible after the transfer of the wild-type beta-globin gene.

The biosafety of LVs has greatly improved in recent years, and there is now very little risk of producing replication-competent lentiviruses [149]. Modifications have included splitting the genetic elements required for production of the viral core proteins, the elimination of accessory genes involved in virus infectivity, the replacement of the viral envelope with that of another class of virus, and the removal of the viral enhancer/promoter elements [150]. Insertional mutagenesis, which led to leukemia in some patients receiving γ-RV transduced cells [151–154], is a major concern when integrative vectors are used. Viral transcriptional activating elements have been shown to trigger oncogene activation, leading to the strong recommendation that these elements should be deleted from retroviral vectors used for gene therapy purposes [155]. LVs have a better safety profile than γ-RVs in hematopoietic cells [156] and are less genotoxic than γ-RVs in transplanted mice [157,158], due to the differential DNA targeting of the two vectors [159]. Nevertheless, LVs in general [159], and β-globin-LVs in particular [160], preferentially integrate into active genes and disturb gene expression over large distances [161]. Furthermore, the level of β-globin gene expression under the control of LCR enhancers is subject to position effects [162,163]. Following vector integration, levels of β-globin expression have been shown to be variable and to depend on the location of the vector in the chromatin in transplantation experiments in mice [164]. Chromatin insulators placed between enhancers and promoters prevent interactions between these elements. They also buffer transgenes against chromosomal position effects [165,166]. Chromatin insulators derived from the chicken hypersensitive site-4 (cHS4) of the β-globin locus [166] were thus introduced into β-globin LVs, to increase efficacy and safety [148,167]. This modification led to a higher probability of β-globin expression and lower levels of position effect variegation *in vitro* [167].

A LV encoding the mutated β-globin (β^{A-T87Q}) was thus constructed. In this vector, referred to as LentiGlobin vector HPV569, the β-globin gene is under the control of the LCR and the β-globin promoter, and is surrounded by tandem copies of the cHS4 core element [148]. Experiments in beta-thalassemia mice subjected to busulfan conditioning and transplantation with cells transduced with this vector showed them to have near-normal hemoglobin levels and to display no overall toxicity [168]. Over the following few years, we performed a large body of experiments with the aim of applying to carry out a clinical trial. This involved efficacy and safety/toxicology studies in mice, and large-scale vector manufacture in good manufacturing practice (GMP) conditions [148,168–170]. Specific qualified assays for quality assurance (QA)/quality control

(QC) were also developed and approved by the regulatory authorities. The Sadelain group and several other groups also worked towards clinical trial applications [171–173]. The differences between our own product and trial protocol and those of the Sadelain group include (1) differences in the boundaries of the beta-globin gene, beta-globin promoter and LCR elements, (2) the initial presence of the cHS4 insulator core in our vector, (3) an anti-sickling and chromatography-identifiable β^{A-T87Q}-globin variant, (4) differences in vector manufacturing and purification, (5) full-dose intravenous busulfan treatment, but without the addition of an immunosuppressive agent, (6) applicability to both beta-thalassemia and sickle cell disease due to our choice of the β^{A-T87Q}-variant, and (7) initial focus on betaE/beta0-thalassemia major patients [148,169,170,174]. We were granted authorization to carry out clinical trials for both beta-thalassemia and sickle cell disease in 2006 (the LG001 Study), by the French regulatory authority ANSM (*Agence Nationale de Sécurité du Médicament et des Produits de Santé*). In these trials, we used the GMP-grade HPV569 vector produced and controlled in the USA. This work [170], together with a clinical trial of gene therapy for adrenoleukodystrophy [175], constituted the first trial of the use of a LV for the treatment of an inborn genetic disease to be approved anywhere in the world.

The first beta-thalassemia patient to be treated and who did not receive backup cells was an 18-year-old β^E/β^0 adult with clinical beta-thalassemia major who had been transfusion-dependent and on parenteral iron chelation therapy since early childhood and had undergone splenectomy at the age of six years, and for whom no HLA-MSD was available [169,170,174]. Bone marrow cells were processed for $CD34^+$ cell selection, transduced with the HPV569 lentiviral vector and thawed for release testing. The patient received an infusion of 3.9×10^6 cells/kg after busulfan conditioning at the fully myeloablative dose (12.8 mg/kg). Hematopoietic reconstitution was uneventful, and the patient became transfusion-independent one year after transplantation. Hemoglobin levels are stable, at about 8–9 g/dL 6.5 years later or 7.5 years since gene therapy, with a mean vector copy number close to 0.2 vector copy per myeloid cell [176]. The transgenic hemoglobin accounts for one third of total hemoglobin (HbF, HbE and HbA^{T87Q}). A number of transduced hematopoietic clones were detected among the lymphoid and myeloid cells. One dominant clone emerged in month 3 and stabilized around month 15. This clone accounted for less than 4% of the peripheral nucleated cells, and this percentage had fallen to less than 2% five years post-transplantation. In this clone, the vector was inserted at the high-mobility group AT-hook 2 (HMGA2) locus, compromising gene splicing and causing the premature termination of transcription. The compromised HMGA2 gene displayed transcriptional activation, generating large amounts of a truncated and active form of the protein in transduced cells. It was suggested, but not formally demonstrated, that there was a causal link between vector insertion at this locus, HMGA2 gene activation, and clonal dominance [170]. High-performance liquid chromatography (HPLC) analysis of single erythroid colonies indicated that vector-derived β^{A-T87Q}-globin expression output did not differ significantly between the HMGA2 integrant and colonies displaying integration at other chromosomal sites [170]. Almost eight years after transplantation, there is no signs of clonal overgrowth or toxicity [176]. Another patient underwent transplantation in November 2011, but the vector copy number was lower. This patient is still transfusion-dependent, and transgenic hemoglobin levels account for ≈5% of total hemoglobin [176].

The first two clinical studies in which LVs were used to transduce HSCs for the treatment of β-thalassemia [170] and adrenoleukodystrophy [175] showed that one key restriction to fully effective treatment was the limited efficacy of stem cell transduction, which was only 10–20%. Other studies suggested that the protection provided by the cHS4 elements was variable [155,177], and dependent on their position in the chromatin [178]. Furthermore, the tandem copies of the cHS4 insulator inserted into the β-globin vector were prone to rearrangement, resulting in the regeneration of a single element upon vector insertion [168,179]. This single element had a lower level of enhancer-blocking activity than the tandem copies [180]. The insertion of insulators into the vector was also shown to decrease vector titer [179,181]. It therefore seemed likely that the removal of the cHS4 elements from the vector would result in a LV with a higher transduction efficacy and a similar level of safety. We therefore simply removed cHS4 from LentiGlobin vector HPV569 to yield LentiGlobin vector BB305, which also contains a heterologous promoter to increase vector titers and yield [182]. The production, concentration and purification protocols were improved [183]. The new vector BB305 was compared with HPV569 in human cells and in a comprehensive mouse toxicology study [182]. It was found to be more effective than the previous vector, with an equivalent and satisfactory level of safety [182]. On the basis of these new preclinical results with LentiGlobin vector BB305 and the sustained positive outcome for a patient with the parental LentiGlobin vector HPV569 [170], both the ANSM (French) and the RAC/FDA (Recombinant DNA Advisory Committee/Federal Drug Administration, US) granted us approval, in 2013 and 2014, for three new clinical trials using LentiGlobin vector BB305 sponsored by the biotechnology company bluebird bio: (1) Trial NCT02151526 (HGB-205) in France, for both beta-thalassemia and sickle cell disease, (2) Trial NCT01745120 (HGB-204) at multiple centers in the US, Australia and Thailand, for beta-thalassemia, and (3) Trial NCT02140554 (HGB-206), at multiple centers in the US, for sickle cell disease. The three trials follow essentially the same clinical transduction protocol as for the previous trial with LentiGlobin vector HPV569, although the BB305 vector is further purified by preparative chromatography.

Very encouraging results have been obtained for transfusion-dependent beta-thalassemia major patients, and were presented by the corresponding clinical investigators at the annual meeting of the American Society of Hematology (ASH) in December 2014 [184,185]. For the HGB-205 trial, transplantation was carried out in two transfusion-dependent β^E/β^0 patients. Six months post-treatment, the mean vector copy numbers per cell were above 1 in peripheral blood, and Hb levels had reached 10.2 and 13.4 g/dL, respectively, in the absence of transfusion, with ≈70% HbA^{T87Q}, ≈30% HbE and low HbF. The two patients became transfusion-independent 10 and 12 days after cell infusion. $CD34^+$ cells from a patient

with sickle cell disease were also transduced with highly efficiently, but clinical data were not available in December 2014. For the HGB-204 study, five transfusion-dependent beta-thalassemia major patients underwent transplantation. Two of these patients were treated more than three months before the data were released in December 2014. One of these patients is β^E/β^0, whereas the other has the Cooley's anemia β^0/β^0 genotype. Both these patients rapidly became transfusion-independent, with stable Hb concentrations of 8.6 and 9.6 g/dL in the absence of transfusion, six and three months post-transplantation, respectively. The other 3 patients underwent transplantation in November 2014, and too little time had elapsed for any meaningful conclusions to be drawn at the time of data release in December 2014. None of the subjects experienced a drug product-related adverse event in either of these trials. Vector integration site analyses showed polyclonal reconstitution without clonal dominance. Additional subjects were still awaiting transplantation in December 2014.

Another clinical trial, sponsored by the Memorial Sloan-Kettering Cancer Center of New York, USA, was also initiated in 2012, in adult β-thalassemia major patients receiving autologous $CD34^+$ cells, with non-myeloablative conditioning, after transduction with the TNS9.3.55 LV from the Sadelain group (clinicalTrial.gov identifier: NCT01639690) [171,173]. A non-myeloablative conditioning regimen would clearly have advantages over a fully ablative regimen, but such a regime may not currently be sufficient to ensure optimal engraftment with transduced stem cells. The clinical data from this trial have yet to be formally released.

Conclusion

Future treatments for beta-thalassemia will depend on the benefit/risk/cost ratios for conventional transfusion and iron chelation [70], γ-globin induction [186], allogeneic bone marrow transplantation [187] and gene therapy [170], and the availability of these treatments in the low-income countries in which most β-thalassemia patients live. Treatments with HU or with other γ-globin inducers, either alone or in combination, have been shown to increase Hb levels by 1–5 g/dL [188]. However, HU is currently the only compound approved for the treatment of adult patients with sickle cell disease. Furthermore, a number of beta-thalassemia patients fail to achieve significant increases in HbF levels and clinical improvement on this treatment [44,189]. Most patients with beta-thalassemia major continue to suffer from profound anemia [69,190] and new drugs inducing HbF are being investigated [191]. In the field of hematopoietic stem cell transplantation, Investigations are continuing, with the aim of extending allogeneic stem cell infusion to a larger number of patients. The use of haploidentical parent or sibling donors may extend the use of this treatment to patients lacking a MSD or HLA-compatible UD, provided that the balance between the risks of GVHD and graft failure is considered acceptable. Recent successes have been reported in small series of patients [192–194] and require confirmation in larger cohorts, with longer periods of follow-up. Significant improvements in clinical outcomes have been obtained through progress in the domains of conditioning regimens, risk stratification and stem cell sources. However, allogeneic stem cell transplantation remains a complicated and risky procedure. Many of its drawbacks, such as GVHD and GR in particular, may be avoided by the use of autologous stem cell transplantation after corrective gene transfer. Gene therapy is still at an early stage of development, but our clinical trials have already provided proof-of-principle for sustained clinical efficacy with low toxicity in several patients. With improvements in the production of LentiGlobin vector BB305, all of the patients treated rapidly became transfusion-independent, with near-normal Hb concentrations in the blood, even in one patient with β^0/β^0-thalassemia (Cooley's anemia). Gene therapy may thus become an attractive mode of treatment for young adults with no MSD considered to be at low risk of transplant complications. Further studies will be required before this approach can be offered as a first-line medical treatment. For high-risk class 3 patients, reduced and adapted preparative regimens must be tested. Methods for selecting and amplifying transduced stem cells may be required to maximize efficacy in such a context of reduced conditioning [195]. Finally, although recent gene and cell therapy trials involving LV-mediated gene transfer in HSCs followed by autologous cell transplantation gave excellent outcomes, with no observable toxicity [175,184,196,197], it is still too early to draw any firm conclusions about the risk of insertional mutagenesis. Gene vector targeting and gene modification by homologous recombination [198] remain suboptimal, but research is continuing. Combined gene and cell therapy approaches may make it possible to exploit the proliferative potential of pluripotent stem cells, but many obstacles to the use of such approaches for HSCT remain [199,200]. The next few years will probably confirm the enormous potential of gene transfer methods for the treatment of β-thalassemia patients, in terms of both efficacy and safety.

Conflicts of interest

Edouard de Dreuzy and Kanit Bhukhai have no competing interests. Philippe Leboulch and Emmanuel Payen have financial relationships with Bluebird Bio, Inc.

Acknowledgments

The work of the authors is supported by an Industrial Chair from France's ANR (Agence Nationale pour la Recherche) (HemGenTher, ANR-12-CHIN-005-01) awarded to Philippe Leboulch. Emmanuel Payen, Edouard de Dreuzy and Kanit Bhukhai are supported by INSERM (Institut National de la Santé et de la Recherche Médicale), CEA (Commissariat à l'Energie Atomique et aux Recherches Alternatives) and the Industrial Chair HemGenTher, ANR-12-CHIN-005-01, respectively.

REFERENCES

[1] Flint J, Harding RM, Boyce AJ, Clegg JB. The population genetics of the haemoglobinopathies. Baillieres Clin Haematol 1998;11:1–51.

[2] Borgna-Pignatti C, Marsella M, Zanforlin N. The natural history of thalassemia intermedia. Ann N Y Acad Sci 2010;1202:214–20.
[3] Rachmilewitz EA, Giardina PJ. How I treat thalassemia. Blood 2011;118:3479–88.
[4] Borgna-Pignatti C. The life of patients with thalassemia major. Haematologica 2010;95:345–8.
[5] Modell B, Khan M, Darlison M, Westwood MA, Ingram D, Pennell DJ. Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. J Cardiovasc Magn Reson 2008;10:42.
[6] Thuret I, Pondarre C, Loundou A, Steschenko D, Girot R, Bachir D, et al. Complications and treatment of patients with beta-thalassemia in France: results of the National Registry. Haematologica 2010;95:724–9.
[7] Thein SL, Craig JE. Genetics of Hb F/F cell variance in adults and heterocellular hereditary persistence of fetal hemoglobin. Hemoglobin 1998;22:401–14.
[8] Thein SL, Wood WG. The molecular basis of b thalassemia, db thalassemia and hereditary persistence of fetal hemoglobin. In: Steinberg MH, Forget BG, Higgs DR, Weatherall DJ, editors. Disorders of hemoglobin. Cambridge, UK: Cambridge University; 2009. p. 323–56.
[9] Garner C, Tatu T, Reittie JE, Littlewood T, Darley J, Cervino S, et al. Genetic influences on F cells and other hematologic variables: a twin heritability study. Blood 2000;95:342–6.
[10] Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, Wang H, et al. Systematic localization of common disease-associated variation in regulatory DNA. Science 2012;337:1190–5.
[11] Lettre G, Sankaran VG, Bezerra MA, Araujo AS, Uda M, Sanna S, et al. DNA polymorphisms at the BCL11A, HBS1L-MYB, and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. Proc Natl Acad Sci U S A 2008;105:11869–74.
[12] Menzel S, Garner C, Gut I, Matsuda F, Yamaguchi M, Heath S, et al. A QTL influencing F cell production maps to a gene encoding a zinc-finger protein on chromosome 2p15. Nat Genet 2007;39:1197–9.
[13] Nuinoon M, Makarasara W, Mushiroda T, Setianingsih I, Wahidiyat PA, Sripichai O, et al. A genome-wide association identified the common genetic variants influence disease severity in beta0-thalassemia/hemoglobin E. Hum Genet 2010;127:303–14.
[14] Uda M, Galanello R, Sanna S, Lettre G, Sankaran VG, Chen W, et al. Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of beta-thalassemia. Proc Natl Acad Sci U S A 2008;105:1620–5.
[15] Thein SL, Menzel S, Peng X, Best S, Jiang J, Close J, et al. Intergenic variants of HBS1L-MYB are responsible for a major quantitative trait locus on chromosome 6q23 influencing fetal hemoglobin levels in adults. Proc Natl Acad Sci U S A 2007;104:11346–51.
[16] Farrell JJ, Sherva RM, Chen ZY, Luo HY, Chu BF, Ha SY, et al. A 3-bp deletion in the HBS1L-MYB intergenic region on chromosome 6q23 is associated with HbF expression. Blood 2011;117:4935–45.
[17] Borg J, Papadopoulos P, Georgitsi M, Gutierrez L, Grech G, Fanis P, et al. Haploinsufficiency for the erythroid transcription factor KLF1 causes hereditary persistence of fetal hemoglobin. Nat Genet 2010;42:801–5.
[18] Solovieff N, Milton JN, Hartley SW, Sherva R, Sebastiani P, Dworkis DA, et al. Fetal hemoglobin in sickle cell anemia: genome-wide association studies suggest a regulatory region in the 5′ olfactory receptor gene cluster. Blood 2010;115:1815–22.
[19] Bae HT, Baldwin CT, Sebastiani P, Telen MJ, Ashley-Koch A, Garrett M, et al. Meta-analysis of 2040 sickle cell anemia patients: BCL11A and HBS1L-MYB are the major modifiers of HbF in African Americans. Blood 2012;120:1961–2.
[20] Cardoso GL, Diniz IG, Silva AN, Cunha DA, Silva Junior JS, Uchoa CT, et al. DNA polymorphisms at BCL11A, HBS1L-MYB and Xmn1-HBG2 site loci associated with fetal hemoglobin levels in sickle cell anemia patients from Northern Brazil. Blood Cells Mol Dis 2014;53:176–9.
[21] Makani J, Menzel S, Nkya S, Cox SE, Drasar E, Soka D, et al. Genetics of fetal hemoglobin in Tanzanian and British patients with sickle cell anemia. Blood 2011;117:1390–2.
[22] Nguyen TK, Joly P, Bardel C, Moulsma M, Bonello-Palot N, Francina A. The XmnI (G)gamma polymorphism influences hemoglobin F synthesis contrary to BCL11A and HBS1L-MYB SNPs in a cohort of 57 beta-thalassemia intermedia patients. Blood Cells Mol Dis 2010;45:124–7.
[23] Wonkam A, Ngo Bitoungui VJ, Vorster AA, Ramesar R, Cooper RS, Tayo B, et al. Association of variants at BCL11A and HBS1L-MYB with hemoglobin F and hospitalization rates among sickle cell patients in Cameroon. PLoS One 2014;9:e92506.
[24] Sampietro M, Thein SL, Contreras M, Pazmany L. Variation of HbF and F-cell number with the G-gamma Xmn I (C-T) polymorphism in normal individuals. Blood 1992;79:832–3.
[25] Leonova J, Kazanetz EG, Smetanina NS, Adekile AD, Efremov GD, Huisman TH. Variability in the fetal hemoglobin level of the normal adult. Am J Hematol 1996;53:59–65.
[26] Labie D, Pagnier J, Lapoumeroulie C, Rouabhi F, Dunda-Belkhodja O, Chardin P, et al. Common haplotype dependency of high G gamma-globin gene expression and high Hb F levels in beta-thalassemia and sickle cell anemia patients. Proc Natl Acad Sci U S A 1985;82:2111–4.
[27] Gilman JG, Huisman TH. DNA sequence variation associated with elevated fetal G gamma globin production. Blood 1985;66:783–7.
[28] Neishabury M, Zamani S, Azarkeivan A, Abedini SS, Darvish H, Zamani F, et al. The modifying effect of Xmn1-HBG2 on thalassemic phenotype is associated with its linked elements in the beta globin locus control region, including the palindromic site at 5′HS4. Blood Cells Mol Dis 2012;48:1–5.
[29] Weatherall DJ. Commentary on "The modifying effect of Xmn1-HBG2 on thalassemic phenotype is associated with its linked elements in the beta globin locus control region, including the palindromic site at 5′ HS4" by M. Neishabury et al. Blood Cells Mol Dis 2012;48:6.
[30] Neishabury M, Zamani F, Keyhani E, Azarkeivan A, Abedini SS, Eslami MS, et al. The influence of the BCL11A polymorphism on the phenotype of patients with beta thalassemia could be affected by the beta globin locus control region and/or the Xmn1-HBG2 genotypic background. Blood Cells Mol Dis 2013;51:80–4.
[31] Sankaran VG, Xu J, Ragoczy T, Ippolito GC, Walkley CR, Maika SD, et al. Developmental and species-divergent globin switching are driven by BCL11A. Nature 2009;460:1093–7.
[32] Wilber A, Hargrove PW, Kim YS, Riberdy JM, Sankaran VG, Papanikolaou E, et al. Therapeutic levels of fetal hemoglobin in erythroid progeny of beta-thalassemic CD34+ cells after lentiviral vector-mediated gene transfer. Blood 2011;117:2817–26.

[33] Bauer DE, Kamran SC, Lessard S, Xu J, Fujiwara Y, Lin C, et al. An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. Science 2013;342:253–7.
[34] Zhou D, Liu K, Sun CW, Pawlik KM, Townes TM. KLF1 regulates BCL11A expression and gamma- to beta-globin gene switching. Nat Genet 2010;42:742–4.
[35] Satta S, Perseu L, Moi P, Asunis I, Cabriolu A, Maccioni L, et al. Compound heterozygosity for KLF1 mutations associated with remarkable increase of fetal hemoglobin and red cell protoporphyrin. Haematologica 2011;96:767–70.
[36] Vegiopoulos A, Garcia P, Emambokus N, Frampton J. Coordination of erythropoiesis by the transcription factor c-Myb. Blood 2006;107:4703–10.
[37] Sankaran VG, Menne TF, Scepanovic D, Vergilio JA, Ji P, Kim J, et al. MicroRNA-15a and -16-1 act via MYB to elevate fetal hemoglobin expression in human trisomy 13. Proc Natl Acad Sci U S A 2011;108:1519–24.
[38] Jiang J, Best S, Menzel S, Silver N, Lai MI, Surdulescu GL, et al. cMYB is involved in the regulation of fetal hemoglobin production in adults. Blood 2006;108:1077–83.
[39] Wahlberg K, Jiang J, Rooks H, Jawaid K, Matsuda F, Yamaguchi M, et al. The HBS1L-MYB intergenic interval associated with elevated HbF levels shows characteristics of a distal regulatory region in erythroid cells. Blood 2009;114:1254–62.
[40] Stadhouders R, Aktuna S, Thongjuea S, Aghajanirefah A, Pourfarzad F, van Ijcken W, et al. HBS1L-MYB intergenic variants modulate fetal hemoglobin via long-range MYB enhancers. J Clin Invest 2014;124:1699–710.
[41] Koehler AN. A complex task? Direct modulation of transcription factors with small molecules. Curr Opin Chem Biol 2010;14:331–40.
[42] Yu Y, Wang J, Khaled W, Burke S, Li P, Chen X, et al. Bcl11a is essential for lymphoid development and negatively regulates p53. J Exp Med 2012;209:2467–83.
[43] Lin J, Chen H, Luo L, Lai Y, Xie W, Kee K. Creating a monomeric endonuclease TALE-I-SceI with high specificity and low genotoxicity in human cells. Nucleic Acids Res 2015;43:1112–22.
[44] Voskaridou E, Christoulas D, Bilalis A, Plata E, Varvagiannis K, Stamatopoulos G, 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.
[45] Ware RE, Aygun B. Advances in the use of hydroxyurea. Hematol Am Soc Hematol Educ Progr 2009:62–9.
[46] Yavarian M, Karimi M, Bakker E, Harteveld CL, Giordano PC. Response to hydroxyurea treatment in Iranian transfusion-dependent beta-thalassemia patients. Haematologica 2004;89:1172–8.
[47] Italia KY, Jijina FF, Merchant R, Panjwani S, Nadkarni AH, Sawant PM, et al. Effect of hydroxyurea on the transfusion requirements in patients with severe HbE-beta-thalassaemia: a genotypic and phenotypic study. J Clin Pathol 2010;63:147–50.
[48] Dixit A, Chatterjee TC, Mishra P, Choudhry DR, Mahapatra M, Tyagi S, et al. Hydroxyurea in thalassemia intermedia–a promising therapy. Ann Hematol 2005;84:441–6.
[49] Alebouyeh M, Moussavi F, Haddad-Deylami H, Vossough P. Hydroxyurea in the treatment of major beta-thalassemia and importance of genetic screening. Ann Hematol 2004;83:430–3.
[50] Bradai M, Pissard S, Abad MT, Dechartres A, Ribeil JA, Landais P, et al. Decreased transfusion needs associated with hydroxyurea therapy in Algerian patients with thalassemia major or intermedia. Transfusion 2007;47:1830–6.
[51] Italia KY, Jijina FJ, Merchant R, Panjwani S, Nadkarni AH, Sawant PM, et al. Response to hydroxyurea in beta thalassemia major and intermedia: experience in western India. Clin Chim Acta 2009;407:10–5.
[52] Karimi M, Haghpanah S, Farhadi A, Yavarian M. Genotype-phenotype relationship of patients with beta-thalassemia taking hydroxyurea: a 13-year experience in Iran. Int J Hematol 2012;95:51–6.
[53] Banan M, Bayat H, Azarkeivan A, Mohammadparast S, Kamali K, Farashi S, et al. The XmnI and BCL11A single nucleotide polymorphisms may help predict hydroxyurea response in Iranian beta-thalassemia patients. Hemoglobin 2012;36:371–80.
[54] Kohne E. Hemoglobinopathies: clinical manifestations, diagnosis, and treatment. Dtsch Arztebl Int 2011;108:532–40.
[55] Castro O, Nouraie M, Oneal P. Hydroxycarbamide treatment in sickle cell disease: estimates of possible leukaemia risk and of hospitalization survival benefit. Br J Haematol 2014;167:687–91.
[56] Sankaran VG, Menne TF, Xu J, Akie TE, Lettre G, Van Handel B, et al. Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. Science 2008;322:1839–42.
[57] Sedgewick AE, Timofeev N, Sebastiani P, So JC, Ma ES, Chan LC, et al. BCL11A is a major HbF quantitative trait locus in three different populations with beta-hemoglobinopathies. Blood Cells Mol Dis 2008;41:255–8.
[58] Flanagan JM, Steward S, Howard TA, Mortier NA, Kimble AC, Aygun B, et al. Hydroxycarbamide alters erythroid gene expression in children with sickle cell anaemia. Br J Haematol 2012;157:240–8.
[59] Ware RE, Despotovic JM, Mortier NA, Flanagan JM, He J, Smeltzer MP, et al. Pharmacokinetics, pharmacodynamics, and pharmacogenetics of hydroxyurea treatment for children with sickle cell anemia. Blood 2011;118:4985–91.
[60] Sheehan VA, Luo Z, Flanagan JM, Howard TA, Thompson BW, Wang WC, et al. Genetic modifiers of sickle cell anemia in the BABY HUG cohort: influence on laboratory and clinical phenotypes. Am J Hematol 2013;88:571–6.
[61] Fanis P, Kousiappa I, Phylactides M, Kleanthous M. Genotyping of BCL11A and HBS1L-MYB SNPs associated with fetal haemoglobin levels: a SNaPshot minisequencing approach. BMC Genom 2014;15:108.
[62] Gravia A, Chondrou V, Sgourou A, Papantoni I, Borg J, Katsila T, et al. Individualizing fetal hemoglobin augmenting therapy for beta-type hemoglobinopathies patients. Pharmacogenomics 2014;15:1355–64.
[63] Pourfarzad F, von Lindern M, Azarkeivan A, Hou J, Kia SK, Esteghamat F, et al. Hydroxyurea responsiveness in beta-thalassemic patients is determined by the stress response adaptation of erythroid progenitors and their differentiation propensity. Haematologica 2013;98:696–704.
[64] Perrine SP. Fetal globin induction–can it cure beta thalassemia? Hematol Am Soc Hematol Educ Progr 2005:38–44.
[65] Kiefer CM, Hou C, Little JA, Dean A. Epigenetics of beta-globin gene regulation. Mutat Res 2008;647:68–76.
[66] Mabaera R, Richardson CA, Johnson K, Hsu M, Fiering S, Lowrey CH. Developmental- and differentiation-specific patterns of human gamma- and beta-globin promoter DNA methylation. Blood 2007;110:1343–52.

[67] Yin W, Barkess G, Fang X, Xiang P, Cao H, Stamatoyannopoulos G, et al. Histone acetylation at the human beta-globin locus changes with developmental age. Blood 2007;110:4101–7.
[68] Paciaroni K, Lucarelli G, Martelli F, Migliaccio AR, von Lindern M, Borg J, et al. Transfusion-independent beta(0)-thalassemia after bone marrow transplantation failure: proposed involvement of high parental HbF and an epigenetic mechanism. Am J Blood Res 2014;4:27–32.
[69] Testa U. Fetal hemoglobin chemical inducers for treatment of hemoglobinopathies. Ann Hematol 2009;88:505–28.
[70] Angelucci E, Barosi G, Camaschella C, Cappellini MD, Cazzola M, Galanello R, et al. Italian society of hematology practice guidelines for the management of iron overload in thalassemia major and related disorders. Haematologica 2008;93:741–52.
[71] Thomas ED, Buckner CD, Sanders JE, Papayannopoulou T, Borgna-Pignatti C, De Stefano P, et al. Marrow transplantation for thalassaemia. Lancet 1982;2:227–9.
[72] Lucarelli G, Polchi P, Galimberti M, Izzi T, Delfini C, Manna M, et al. Marrow transplantation for thalassaemia following busulphan and cyclophosphamide. Lancet 1985;1:1355–7.
[73] Jagannath VA, Fedorowicz Z, Al Hajeri A, Sharma A. Hematopoietic stem cell transplantation for people with ss-thalassaemia major. Cochrane Database Syst Rev 2014;10. CD008708.
[74] La Nasa G, Caocci G, Efficace F, Dessi C, Vacca A, Piras E, et al. Long-term health-related quality of life evaluated more than 20 years after hematopoietic stem cell transplantation for thalassemia. Blood 2013;122:2262–70.
[75] Di Bartolomeo P, Santarone S, Di Bartolomeo E, Olioso P, Bavaro P, Papalinetti G, et al. Long-term results of survival in patients with thalassemia major treated with bone marrow transplantation. Am J Hematol 2008;83:528–30.
[76] Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baronciani D, Giardini C, et al. Bone marrow transplantation in patients with thalassemia. N Engl J Med 1990;322:417–21.
[77] Lucarelli G, Weatherall DJ. For debate: bone marrow transplantation for severe thalassaemia (1). The view from Pesaro (2). To be or not to be. Br J Haematol 1991;78:300–3.
[78] Giardini C, Galimberti M, Lucarelli G. Bone marrow transplantation in thalassemia. Annu Rev Med 1995;46:319–30.
[79] Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baronciani D, Durazzi SM, et al. A new approach to bone marrow transplantation in thalassemia. Ann N. Y Acad Sci 1990;612:394–7.
[80] Lucarelli G, Clift RA, Galimberti M, Polchi P, Angelucci E, Baronciani D, et al. Marrow transplantation for patients with thalassemia: results in class 3 patients. Blood 1996;87:2082–8.
[81] Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baronciani D, Durazzi SM, et al. Bone marrow transplantation in adult thalassemia. Blood 1992;80:1603–7.
[82] Lucarelli G, Clift RA, Galimberti M, Angelucci E, Giardini C, Baronciani D, et al. Bone marrow transplantation in adult thalassemic patients. Blood 1999;93:1164–7.
[83] Isgro A, Gaziev J, Sodani P, Lucarelli G. Progress in hematopoietic stem cell transplantation as allogeneic cellular gene therapy in thalassemia. Ann N Y Acad Sci 2010;1202:149–54.
[84] Mathews V, George B, Deotare U, Lakshmi KM, Viswabandya A, Daniel D, et al. A new stratification strategy that identifies a subset of class III patients with an adverse prognosis among children with beta thalassemia major undergoing a matched related allogeneic stem cell transplantation. Biol Blood Marrow Transpl 2007;13:889–94.
[85] Sabloff M, Chandy M, Wang Z, Logan BR, Ghavamzadeh A, Li CK, et al. HLA-matched sibling bone marrow transplantation for beta-thalassemia major. Blood 2011;117:1745–50.
[86] Hongeng S, Pakakasama S, Chuansumrit A, Sirachainan N, Sura T, Ungkanont A, et al. Reduced intensity stem cell transplantation for treatment of class 3 Lucarelli severe thalassemia patients. Am J Hematol 2007;82:1095–8.
[87] Choudhary D, Sharma SK, Gupta N, Kharya G, Pavecha P, Handoo A, et al. Treosulfan-thiotepa-fludarabine-based conditioning regimen for allogeneic transplantation in patients with thalassemia major: a single-center experience from north India. Biol Blood Marrow Transpl 2013;19:492–5.
[88] Bernardo ME, Piras E, Vacca A, Giorgiani G, Zecca M, Bertaina A, et al. Allogeneic hematopoietic stem cell transplantation in thalassemia major: results of a reduced-toxicity conditioning regimen based on the use of treosulfan. Blood 2012;120:473–6.
[89] Mathews V, George B, Viswabandya A, Abraham A, Ahmed R, Ganapule A, et al. Improved clinical outcomes of high risk beta thalassemia major patients undergoing a HLA matched related allogeneic stem cell transplant with a treosulfan based conditioning regimen and peripheral blood stem cell grafts. PLoS One 2013;8:e61637.
[90] Sodani P, Gaziev D, Polchi P, Erer B, Giardini C, Angelucci E, et al. New approach for bone marrow transplantation in patients with class 3 thalassemia aged younger than 17 years. Blood 2004;104:1201–3.
[91] Lucarelli G, Gaziev J. Advances in the allogeneic transplantation for thalassemia. Blood Rev 2008;22:53–63.
[92] Gaziev J, Sodani P, Polchi P, Andreani M, Lucarelli G. Bone marrow transplantation in adults with thalassemia: treatment and long-term follow-up. Ann N Y Acad Sci 2005;1054:196–205.
[93] Goussetis E, Peristeri I, Kitra V, Vessalas G, Paisiou A, Theodosaki M, 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 Transpl 2012;47:1061–6.
[94] Galambrun C, Pondarre C, Bertrand Y, Loundou A, Bordigoni P, Frange P, et al. French multicenter 22-year experience in stem cell transplantation for beta-thalassemia major: lessons and future directions. Biol Blood Marrow Transpl 2013;19:62–8.
[95] Iravani M, Tavakoli E, Babaie MH, Ashouri A, Khatami F, Ghavamzadeh A. Comparison of peripheral blood stem cell transplant with bone marrow transplant in class 3 thalassemic patients. Exp Clin Transpl 2010;8:66–73.
[96] Tiercy JM, Claas F. Impact of HLA diversity on donor selection in organ and stem cell transplantation. Hum Hered 2013;76:178–86.
[97] Pidala J, Kim J, Schell M, Lee SJ, Hillgruber R, Nye V, et al. Race/ethnicity affects the probability of finding an HLA-A, -B, -C and -DRB1 allele-matched unrelated donor and likelihood of subsequent transplant utilization. Bone Marrow Transpl 2013;48:346–50.
[98] Yakoub-Agha I, Mesnil F, Kuentz M, Boiron JM, Ifrah N, Milpied N, et al. Allogeneic marrow stem-cell transplantation from human leukocyte antigen-identical siblings versus human leukocyte antigen-allelic-matched unrelated donors (10/10) in patients with standard-risk hematologic malignancy: a prospective study from the

French Society of Bone Marrow Transplantation and Cell Therapy. J Clin Oncol 2006;24:5695–702.
[99] La Nasa G, Caocci G, Argiolu F, Giardini C, Locatelli F, Vacca A, et al. Unrelated donor stem cell transplantation in adult patients with thalassemia. Bone Marrow Transpl 2005;36:971–5.
[100] Li C, Wu X, Feng X, He Y, Liu H, Pei F, et al. A novel conditioning regimen improves outcomes in beta-thalassemia major patients using unrelated donor peripheral blood stem cell transplantation. Blood 2012;120:3875–81.
[101] Anurathapan U, Pakakasama S, Mekjaruskul P, Sirachainan N, Songdej D, Chuansumrit A, et al. Outcomes of thalassemia patients undergoing hematopoietic stem cell transplantation by using a standard myeloablative versus a novel reduced-toxicity conditioning regimen according to a new risk stratification. Biol Blood Marrow Transpl 2014;20:2066–71.
[102] Anurathapan U, Pakakasama S, Rujkijyanont P, Sirachainan N, Songdej D, Chuansumrit A, et al. Pretransplant immunosuppression followed by reduced-toxicity conditioning and stem cell transplantation in high-risk thalassemia: a safe approach to disease control. Biol Blood Marrow Transpl 2013;19:1259–62.
[103] Lisini D, Zecca M, Giorgiani G, Montagna D, Cristantielli R, Labirio M, et al. Donor/recipient mixed chimerism does not predict graft failure in children with beta-thalassemia given an allogeneic cord blood transplant from an HLA-identical sibling. Haematologica 2008;93:1859–67.
[104] Locatelli F, Rocha V, Reed W, Bernaudin F, Ertem M, Grafakos S, et al. Related umbilical cord blood transplantation in patients with thalassemia and sickle cell disease. Blood 2003;101:2137–43.
[105] Locatelli F, Kabbara N, Ruggeri A, Ghavamzadeh A, Roberts I, Li CK, et al. Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling. Blood 2013;122:1072–8.
[106] Wagner JE, Barker JN, DeFor TE, Baker KS, Blazar BR, Eide C, et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood 2002;100:1611–8.
[107] Rocha V, Wagner Jr JE, Sobocinski KA, Klein JP, Zhang MJ, Horowitz MM, et al. Graft-versus-host disease in children who have received a cord-blood or bone marrow transplant from an HLA-identical sibling. Eurocord and International Bone Marrow Transplant Registry Working Committee on Alternative Donor and Stem Cell Sources. N Engl J Med 2000;342:1846–54.
[108] Ruggeri A, Eapen M, Scaravadou A, Cairo MS, Bhatia M, Kurtzberg J, et al. Umbilical cord blood transplantation for children with thalassemia and sickle cell disease. Biol Blood Marrow Transpl 2011;17:1375–82.
[109] Jaing TH, Hung IJ, Yang CP, Chen SH, Chung HT, Tsay PK, et al. Unrelated cord blood transplantation for thalassaemia: a single-institution experience of 35 patients. Bone Marrow Transpl 2012;47:33–9.
[110] Kolata GB, Wade N. Human gene treatment stirs new debate. Science 1980;210:407.
[111] Mercola KE, Stang HD, Browne J, Salser W, Cline MJ. Insertion of a new gene of viral origin into bone marrow cells of mice. Science 1980;208:1033–5.
[112] Mercola KE, Bar-Eli M, Stang HD, Slamon DJ, Cline MJ. Insertion of new genetic information into bone marrow cells of mice: comparison of two selectable genes. Ann N Y Acad Sci 1982;397:272–80.
[113] Wold B, Wigler M, Lacy E, Maniatis T, Silverstein S, Axel R. Introduction and expression of a rabbit beta-globin gene in mouse fibroblasts. Proc Natl Acad Sci U S A 1979;76:5684–8.
[114] Pellicer A, Wigler M, Axel R, Silverstein S. The transfer and stable integration of the HSV thymidine kinase gene into mouse cells. Cell 1978;14:133–41.
[115] Furore over human genetic engineering. New Sci 1980;88:140.
[116] Wade N. UCLA gene therapy racked by friendly fire. Science 1980;210:509–11.
[117] Kollias G, Wrighton N, Hurst J, Grosveld F. Regulated expression of human A gamma-, beta-, and hybrid gamma beta-globin genes in transgenic mice: manipulation of the developmental expression patterns. Cell 1986;46:89–94.
[118] Townes TM, Lingrel JB, Chen HY, Brinster RL, Palmiter RD. Erythroid-specific expression of human beta-globin genes in transgenic mice. EMBO J 1985;4:1715–23.
[119] Behringer RR, Hammer RE, Brinster RL, Palmiter RD, Townes TM. Two 3′ sequences direct adult erythroid-specific expression of human beta-globin genes in transgenic mice. Proc Natl Acad Sci U S A 1987;84:7056–60.
[120] Kollias G, Hurst J, deBoer E, Grosveld F. The human beta-globin gene contains a downstream developmental specific enhancer. Nucleic Acids Res 1987;15:5739–47.
[121] Trudel M, Costantini FA. 3′ enhancer contributes to the stage-specific expression of the human beta-globin gene. Genes Dev 1987;1:954–61.
[122] Bender MA, Gelinas RE, Miller AD. A majority of mice show long-term expression of a human beta-globin gene after retrovirus transfer into hematopoietic stem cells. Mol Cell Biol 1989;9:1426–34.
[123] 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.
[124] Karlsson S, Bodine DM, Perry L, Papayannopoulou T, Nienhuis AW. Expression of the human beta-globin gene following retroviral-mediated transfer into multipotential hematopoietic progenitors of mice. Proc Natl Acad Sci U S A 1988;85:6062–6.
[125] Tuan D, Solomon W, Li Q, London IM. The "beta-like-globin" gene domain in human erythroid cells. Proc Natl Acad Sci U S A 1985;82:6384–8.
[126] Forrester WC, Thompson C, Elder JT, Groudine M. A developmentally stable chromatin structure in the human beta-globin gene cluster. Proc Natl Acad Sci U S A 1986;83:1359–63.
[127] Forrester WC, Takegawa S, Papayannopoulou T, Stamatoyannopoulos G, Groudine M. Evidence for a locus activation region: the formation of developmentally stable hypersensitive sites in globin-expressing hybrids. Nucleic Acids Res 1987;15:10159–77.
[128] Grosveld F, van Assendelft GB, Greaves DR, Kollias G. Position-independent, high-level expression of the human beta-globin gene in transgenic mice. Cell 1987;51:975–85.
[129] Talbot D, Collis P, Antoniou M, Vidal M, Grosveld F, Greaves DR. A dominant control region from the human beta-globin locus conferring integration site-independent gene expression. Nature 1989;338:352–5.
[130] Forrester WC, Novak U, Gelinas R, Groudine M. Molecular analysis of the human beta-globin locus activation region. Proc Natl Acad Sci U S A 1989;86:5439–43.
[131] Leboulch P, Huang GM, Humphries RK, Oh YH, Eaves CJ, Tuan DY, 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.

[132] Sadelain M, Wang CH, Antoniou M, Grosveld F, Mulligan RC. 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.
[133] Raftopoulos H, Ward M, Leboulch P, Bank A. Long-term transfer and expression of the human beta-globin gene in a mouse transplant model. Blood 1997;90:3414–22.
[134] Rivella S, Sadelain M. Genetic treatment of severe hemoglobinopathies: the combat against transgene variegation and transgene silencing. Semin Hematol 1998;35:112–25.
[135] Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, et al. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell 2004;118:149–61.
[136] Naldini L, Blomer U, Gage FH, Trono D, Verma IM. 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.
[137] Naldini L, Blomer U, Gallay P, Ory D, Mulligan R, Gage FH, et al. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 1996;272:263–7.
[138] Kumar M, Keller B, Makalou N, Sutton RE. Systematic determination of the packaging limit of lentiviral vectors. Hum Gene Ther 2001;12:1893–905.
[139] Malim MH, Hauber J, Le SY, Maizel JV, Cullen BR. The HIV-1 rev trans-activator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. Nature 1989;338:254–7.
[140] May C, Rivella S, Callegari J, Heller G, Gaensler KM, Luzzatto L, et al. Therapeutic haemoglobin synthesis in beta-thalassaemic mice expressing lentivirus-encoded human beta-globin. Nature 2000;406:82–6.
[141] Pawliuk R, Westerman KA, Fabry ME, Payen E, Tighe R, Bouhassira EE, et al. Correction of sickle cell disease in transgenic mouse models by gene therapy. Science 2001;294:2368–71.
[142] Hanawa H, Hargrove PW, Kepes S, Srivastava DK, Nienhuis AW, Persons DA. Extended beta-globin locus control region elements promote consistent therapeutic expression of a gamma-globin lentiviral vector in murine beta-thalassemia. Blood 2004;104:2281–90.
[143] Miccio A, Cesari R, Lotti F, Rossi C, Sanvito F, Ponzoni M, et al. In vivo selection of genetically modified erythroblastic progenitors leads to long-term correction of beta-thalassemia. Proc Natl Acad Sci U S A 2008;105:10547–52.
[144] Puthenveetil G, Scholes J, Carbonell D, Qureshi N, Xia P, Zeng L, et al. Successful correction of the human beta-thalassemia major phenotype using a lentiviral vector. Blood 2004;104:3445–53.
[145] Rivella S, May C, Chadburn A, Riviere I, Sadelain M. A novel murine model of Cooley anemia and its rescue by lentiviral-mediated human beta-globin gene transfer. Blood 2003;101:2932–9.
[146] Imren S, Payen E, Westerman KA, Pawliuk R, Fabry ME, Eaves CJ, et al. Permanent and panerythroid correction of murine beta thalassemia by multiple lentiviral integration in hematopoietic stem cells. Proc Natl Acad Sci U S A 2002;99:14380–5. Epub 2002 Oct 21.
[147] Takekoshi KJ, Oh YH, Westerman KW, London IM, Leboulch P. Retroviral transfer of a human beta-globin/delta-globin hybrid gene linked to beta locus control region hypersensitive site 2 aimed at the gene therapy of sickle cell disease. Proc Natl Acad Sci U S A 1995;92:3014–8.
[148] Bank A, Dorazio R, Leboulch P. A phase I/II clinical trial of beta-globin gene therapy for beta-thalassemia. Ann N Y Acad Sci 2005;1054:308–16.
[149] Delenda C, Audit M, Danos O. Biosafety issues in lentivector production. Curr Top Microbiol Immunol 2002;261:123–41.
[150] Sakuma T, Barry MA, Ikeda Y. Lentiviral vectors: basic to translational. Biochem J 2012;443:603–18.
[151] Avedillo Diez I, Zychlinski D, Coci EG, Galla M, Modlich U, Dewey RA, et al. Development of novel efficient SIN vectors with improved safety features for Wiskott-Aldrich syndrome stem cell based gene therapy. Mol Pharm 2011;8:1525–37.
[152] Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest 2008;118:3132–42.
[153] Howe SJ, Mansour MR, Schwarzwaelder K, Bartholomae C, Hubank M, Kempski H, et al. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J Clin Invest 2008;118:3143–50.
[154] Stein S, Ott MG, Schultze-Strasser S, Jauch A, Burwinkel B, Kinner A, et al. Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. Nat Med 2010;16:198–204.
[155] Zychlinski D, Schambach A, Modlich U, Maetzig T, Meyer J, Grassman E, et al. Physiological promoters reduce the genotoxic risk of integrating gene vectors. Mol Ther 2008;16:718–25.
[156] De Palma M, Montini E, Santoni de Sio FR, Benedicenti F, Gentile A, Medico E, et al. Promoter trapping reveals significant differences in integration site selection between MLV and HIV vectors in primary hematopoietic cells. Blood 2005;105:2307–15.
[157] Montini E, Cesana D, Schmidt M, Sanvito F, Bartholomae CC, Ranzani M, et al. The genotoxic potential of retroviral vectors is strongly modulated by vector design and integration site selection in a mouse model of HSC gene therapy. J Clin Invest 2009;119:964–75.
[158] Montini E, Cesana D, Schmidt M, Sanvito F, Ponzoni M, Bartholomae C, et al. Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration. Nat Biotechnol 2006;24:687–96.
[159] Bushman F, Lewinski M, Ciuffi A, Barr S, Leipzig J, Hannenhalli S, et al. Genome-wide analysis of retroviral DNA integration. Nat Rev Microbiol 2005;3:848–58.
[160] Imren S, Fabry ME, Westerman KA, Pawliuk R, Tang P, Rosten PM, et al. High-level beta-globin expression and preferred intragenic integration after lentiviral transduction of human cord blood stem cells. J Clin Invest 2004;114:953–62.
[161] Hargrove PW, Kepes S, Hanawa H, Obenauer JC, Pei D, Cheng C, et al. Globin lentiviral vector insertions can perturb the expression of endogenous genes in beta-thalassemic hematopoietic cells. Mol Ther 2008;16:525–33.
[162] Milot E, Strouboulis J, Trimborn T, Wijgerde M, de Boer E, Langeveld A, et al. Heterochromatin effects on the frequency and duration of LCR-mediated gene transcription. Cell 1996;87:105–14.
[163] Alami R, Greally JM, Tanimoto K, Hwang S, Feng YQ, Engel JD, et al. Beta-globin YAC transgenes exhibit uniform expression levels but position effect variegation in mice. Hum Mol Genet 2000;9:631–6.
[164] Persons DA, Hargrove PW, Allay ER, Hanawa H, Nienhuis AW. 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.

[165] Bell AC, West AG, Felsenfeld G. The protein CTCF is required for the enhancer blocking activity of vertebrate insulators. Cell 1999;98:387–96.
[166] Recillas-Targa F, Pikaart MJ, Burgess-Beusse B, Bell AC, Litt MD, West AG, 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.
[167] Arumugam PI, Scholes J, Perelman N, Xia P, Yee JK, Malik P. 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.
[168] Ronen K, Negre O, Roth S, Colomb C, Malani N, Denaro M, et al. Distribution of lentiviral vector integration sites in mice following therapeutic gene transfer to treat beta-thalassemia. Mol Ther 2011;19:1273–86.
[169] Payen E, Colomb C, Negre O, Beuzard Y, Hehir K, Leboulch P. Lentivirus vectors in beta-thalassemia. Methods Enzymol 2012;507:109–24.
[170] Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, et al. Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. Nature 2010;467:318–22.
[171] Boulad F, Wang X, Qu J, Taylor C, Ferro L, Karponi G, et al. Safe mobilization of CD34+ cells in adults with beta-thalassemia and validation of effective globin gene transfer for clinical investigation. Blood 2014;123:1483–6.
[172] Frittoli MC, Biral E, Cappelli B, Zambelli M, Roncarolo MG, Ferrari G, et al. Bone marrow as a source of hematopoietic stem cells for human gene therapy of beta-thalassemia. Hum Gene Ther 2011;22:507–13.
[173] Sadelain M, Boulad F, Riviere I, Maggio A, Taher A. Gene therapy. In: Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V, editors. Guidelines for the management of transfusion dependent thalassaemia (TDT) [internet]. 3rd ed. Nicosia, Cyprus: Thalassaemia International Federation; 2013.
[174] Payen E, Leboulch P. Advances in stem cell transplantation and gene therapy in the beta-hemoglobinopathies. Hematol Am Soc Hematol Educ Progr 2012;2012:276–83.
[175] Cartier N, Hacein-Bey-Abina S, Bartholomae CC, Veres G, Schmidt M, Kutschera I, et al. Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. Science 2009;326:818–23.
[176] Cavazzana M, Ribeil JA, Payen E, Suarez F, Negre O, Beuzard Y, et al. Outcomes of gene therapy for beta-thalassemai major via transplantation of autologous hematopoietic stem cells transduced ex vivo with a lentiviral beta globin vector. Haematologica 2014;99. abstract S742.
[177] Ryu BY, Evans-Galea MV, Gray JT, Bodine DM, Persons DA, Nienhuis AW. An experimental system for the evaluation of retroviral vector design to diminish the risk for proto-oncogene activation. Blood 2008;111:1866–75.
[178] Desprat R, Bouhassira EE. Gene specificity of suppression of transgene-mediated insertional transcriptional activation by the chicken HS4 insulator. PLoS One 2009;4:e5956.
[179] Hanawa H, Yamamoto M, Zhao H, Shimada T, Persons DA. Optimized lentiviral vector design improves titer and transgene expression of vectors containing the chicken beta-globin locus HS4 insulator element. Mol Ther 2009;17:667–74.
[180] Chung JH, Bell AC, Felsenfeld G. Characterization of the chicken beta-globin insulator. Proc Natl Acad Sci U S A 1997;94:575–80.
[181] Nielsen TT, Jakobsson J, Rosenqvist N, Lundberg C. Incorporating double copies of a chromatin insulator into lentiviral vectors results in less viral integrants. BMC Biotechnol 2009;9:13.
[182] Negre O, Bartholomae C, Beuzard Y, Cavazzana M, Christiansen L, Courne C, et al. Preclinical evaluation of efficacy and safety of an improved lentiviral vector for the treatment of beta-thalassemia and sickle cell disease. Curr Gene Ther 2015;15:64–81.
[183] Kutner RH, Zhang XY, Reiser J. Production, concentration and titration of pseudotyped HIV-1-based lentiviral vectors. Nat Protoc 2009;4:495–505.
[184] Cavazzana M, Ribeil JA, Payen E, Suarez F, Beuzard Y, Touzot F, et al. Study Hgb-205: outcomes of gene therapy for hemoglobinopathies via transplantation of autologous hematopoietic stem cells transduced ex vivo with a lentiviral $\beta^{A\text{-}T87Q}$-globin vector (LentiGlobin BB305 drug product) [abstract]. Blood 2014;124:4797.
[185] Thompson AA, Rasko JE, Hongeng S, Kwiatkowski JL, Schiller G, Von Kalle C, et al. Initial results from the northstar study (HGB-204): a phase 1/2 study of gene therapy for β-thalassemia major via transplantation of autologous hematopoietic stem cells transduced ex vivo with a lentiviral $\beta^{A\text{-}T87Q}$-globin vector (LentiGlobin BB305 drug product) [abstract]. Blood 2014;124:549.
[186] Boosalis MS, Castaneda SA, Trudel M, Mabaera R, White GL, Lowrey CH, et al. Novel therapeutic candidates, identified by molecular modeling, induce gamma-globin gene expression in vivo. Blood Cells Mol Dis 2011;47:107–16.
[187] Angelucci E, Matthes-Martin S, Baronciani D, Bernaudin F, Bonanomi S, Cappellini MD, et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. Haematologica 2014;99:811–20.
[188] Thein SL. The emerging role of fetal hemoglobin induction in non-transfusion-dependent thalassemia. Blood Rev 2012;26(Suppl. 1):S35–9.
[189] Steinberg MH, Barton F, Castro O, Pegelow CH, Ballas SK, Kutlar A, et al. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. JAMA 2003;289:1645–51.
[190] Musallam KM, Taher AT, Cappellini MD, Sankaran VG. Clinical experience with fetal hemoglobin induction therapy in patients with beta-thalassemia. Blood 2013;121:2199–212. quiz 372.
[191] Ronzoni L, Sonzogni L, Fossati G, Modena D, Trombetta E, Porretti L, et al. Modulation of gamma globin genes expression by histone deacetylase inhibitors: an in vitro study. Br J Haematol 2014;165:714–21.
[192] Bertaina A, Merli P, Rutella S, Pagliara D, Bernardo ME, Masetti R, et al. HLA-haploidentical stem cell transplantation after removal of alphabeta+ T and B cells in children with nonmalignant disorders. Blood 2014;124:822–6.
[193] Sodani P, Isgro A, Gaziev J, Paciaroni K, Marziali M, Simone MD, et al. T cell-depleted hla-haploidentical stem cell transplantation in thalassemia young patients. Pediatr Rep 2011;3(Suppl. 2):e13.
[194] Jaing TH, Sun CF, Lee WI, Wen YC, Yang CP, Hung IJ. Successful unmanipulated peripheral blood progenitor cell transplantation from an HLA haploidentical 2-locus-mismatched mother in a thalassemic patient with primary graft failure after transplantation of bone marrow and cord blood from unrelated donors. Pediatr Transpl 2008;12:232–4.
[195] Negre O, Fusil F, Colomb C, Roth S, Gillet-Legrand B, Henri A, et al. Correction of murine beta-thalassemia after minimal lentiviral gene transfer and homeostatic in vivo erythroid expansion. Blood 2011;117:5321–31.

[196] Aiuti A, Biasco L, Scaramuzza S, Ferrua F, Cicalese MP, Baricordi C, et al. Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. Science 2013;341:1233151.
[197] Biffi A, Montini E, Lorioli L, Cesani M, Fumagalli F, Plati T, et al. Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. Science 2013;341:1233158.
[198] Li HL, Nakano T, Hotta A. Genetic correction using engineered nucleases for gene therapy applications. Dev Growth Differ 2014;56:63–77.
[199] Tubsuwan A, Abed S, Deichmann A, Kardel MD, Bartholoma C, Cheung A, et al. Parallel assessment of globin lentiviral transfer in induced pluripotent stem cells and adult hematopoietic stem cells derived from the same transplanted beta-thalassemia patient. Stem Cells 2013;31:1785–94.
[200] Papapetrou EP, Lee G, Malani N, Setty M, Riviere I, Tirunagari LM, et al. Genomic safe harbors permit high beta-globin transgene expression in thalassemia induced pluripotent stem cells. Nat Biotechnol 2011;29:73–8.