Gene Therapy for Hemoglobinopathies

Marina Cavazzana[1,*] and Fulvio Mavilio[1,2,*]

[1]University of Paris Descartes-Sorbonne Paris Cité, IMAGINE Institute, Paris, France; and [2]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy.

Gene therapy for β-thalassemia and sickle-cell disease is based on transplantation of genetically corrected, autologous hematopoietic stem cells. Preclinical and clinical studies have shown the safety and efficacy of this therapeutic approach, currently based on lentiviral vectors to transfer a β-globin gene under the transcriptional control of regulatory elements of the β-globin locus. Nevertheless, a number of factors are still limiting its efficacy, such as limited stem-cell dose and quality, suboptimal gene transfer efficiency and gene expression levels, and toxicity of myeloablative regimens. In addition, the cost and complexity of the current vector and cell manufacturing clearly limits its application to patients living in less favored countries, where hemoglobinopathies may reach endemic proportions. Gene-editing technology may provide a therapeutic alternative overcoming some of these limitations, though proving its safety and efficacy will most likely require extensive clinical investigation.

Keywords: thalassemia; sickle-cell disease; globin genes; lentiviral vectors; gene editing

INTRODUCTION

HEMOGLOBINOPATHIES ARE INHERITED blood disorders characterized by defective synthesis of hemoglobin (Hb) chains or by the synthesis of mutated globin variants, such as the $\beta^{A\text{-}E6V}$ causing sickle-cell diseases (SCD). Approximately 5% of the world population carries a Hb disorder trait, making hemoglobinopathies the most frequent monogenic diseases worldwide.[1] The only therapy for β-thalassemia and SCD is allogeneic hematopoietic stem-cell (HSC) transplantation from human leukocyte antigen (HLA)-matched sibling donors.[2] Matched unrelated or mismatched HSC transplants carry unacceptable risks of morbidity and mortality, given the current high standards of care. Transplantation of autologous, genetically corrected HSCs is a potential therapeutic alternative, carrying lower transplant-related risks and theoretically being available to all patients. Hematopoietic stem/progenitor cells (HSPCs) transduced by lentiviral vectors (LVs) have been used in clinical trials of gene therapy for immunodeficiencies and lysosomal storage disorders, providing strong evidence of safety and long-term efficacy in most cases.[3] In particular. LVs showed relatively safe integration patterns in the human genome and appeared to cause little interference with normal gene regulation and no significant alterations in the homeostasis of the hematopoietic system.[4–6]

Gene therapy for hemoglobinopathies is more challenging. Regulation of globin gene expression is sophisticated and relies on the interaction of gene promoters with a locus-control region (LCR) that promotes high-level, erythroid-restricted transcription and controls the developmental switch of embryonic to fetal to adult globin gene expression.[7,8] The combination of a full LCR and a complete β-globin gene is too large to fit in an LV: current vectors feature size-reduced LCR and non-coding portions of the β-globin gene but remain complex and transduce HSPCs less efficiently compared to simpler vectors, conflicting with the need to engraft a high proportion of transduced HSCs in the patient's bone marrow (BM). The clinical history of allogeneic HSC transplantation indicates that

*Correspondence: Marina Cavazzana, Imagine Institute, 24 Boulevard de Montparnasse, 75015 Paris, France. E-mail: m.cavazzana@aphp.fr; or Fulvio Mavilio, Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi 287, 41100 Modena, Italy. E-mail: fulvio.mavilio@unimore.it

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stable mixed chimerism with as low as 10–30% donor HSCs leads to significant amelioration of both β-thalassemia and SCD pathology, providing predictions about the minimal level of gene-corrected HSCs that may achieve clinical benefit.[9–11] Recent gene therapy clinical trials are confirming these predictions, although the reduced output of a vector-borne transgene with respect to the natural β-globin locus significantly increases the minimal chimerism levels necessary to achieve efficacy, particularly in the case of SCD.[12–14] Innovation in HSC procurement, transduction, and transplantation technology will likely increase the overall efficacy of gene therapy for hemoglobinopathies in the near future.

LVS FOR GENE THERAPY OF HEMOGLOBINOPATHIES

The first β-globin LVs were developed almost 20 years ago. They contained a β-globin gene under the control of the its promoter and 3′ enhancer elements in addition to the DNase I hypersensitive sites 2, 3, and 4 (HS2, HS3, and HS4) of the β-globin LCR.[15,16] These vectors, termed TNS9 and HPV569, expressed levels of β-globin that were sufficient to correct murine models of β-thalassemia[15,17,18] and SCD.[16] The GLOBE vector had a different design, lacking the HS4 and the 3′ enhancer and featuring an extended HS2 and an HS3 element. This vector showed improved infectivity compared to vectors containing the HS4 and was equally efficacious in correcting murine β-thalassemia[19] and reducing globin chain imbalance in erythroblasts from β-thalassemic patients.[20] All globin vectors transduce long-term repopulating HSCs and combine the low genotoxic properties of LVs with the strict lineage specificity of the β-globin promoter, enhancer, and LCR.[21]

Attempts to increase globin gene expression by introducing larger HS fragments or adding the LCR HS1 element in the LV provided at best modest improvements.[22,23] Other investigators added chromatin "insulators," such as the chicken β-globin HS4[22,24] or a synthetic element (FB) containing sequences from the chicken HS4 and the T-cell receptor BEAD-1 insulator, in an attempt to prevent repressive influence of surrounding chromatin on the integrated vector or reduce potential cis-acting influences of the LCR enhancers on neighboring genes.[25,26] Unfortunately, these elements reduced transduction efficiency and caused genetic instability. In the first patient who received gene therapy for β-thalassemia, the chicken HS4 enhancer in the BGI vector contained a cryptic signal that triggered abnormal splicing of the proto-oncogene HMG2A and a benign clonal dominance.[12] The element was removed for further clinical development.[27] Other attempts to introduce chromatin remodeling elements, such as HS elements derived from the GATA-1 transcription factor gene[28] or the ankyrin gene insulator,[29,30] did improve vector expression features, indicating that combinations of erythroid-specific transcriptional regulatory elements may lead to more efficacious globin LVs. Nevertheless, the vectors currently in clinical development, such as BB305[21] and GLOBE,[19] contain just the essential promoter and LCR elements of the β-globin gene.

While a simple gene-replacement strategy may be sufficient to correct β-thalassemia, vectors aimed at correcting SCD must express globins able to compete with the β^S-chain for α-chains and therefore interfere with Hb polymerization. The design of anti-sickling globins is based on genetic knowledge. SCD patients with elevated levels of fetal Hb (HbF), due to association with a hereditary persistence of HbF (HPFH) trait, experience less severe symptoms.[31,32] The protective activity of HbF is due to the polymerization-inhibiting properties of γ-globin chains, which are able to inhibit sickling. Accordingly, LVs containing a γ-globin or a hybrid β-promoter/γ-globin gene efficiently corrected a murine model of SCD.[33,34] Some investigators developed β-globins carrying mutations that interfere with axial and lateral contacts in the HbS polymer, with potent anti-sickling properties.[35] The BB305 vector expresses one of these mutants, the $\beta^{A\text{-}T87Q}$ globin, and is able to reduce sickling in a murine model of SCD.[16] Another such mutant, the AS3 globin, carries three mutations—T87Q, G16D, and E22A—which further reduce polymerization while increasing the affinity for the α-chain.[36] The βAS3-FB vector, which expresses AS3 globin, corrects murine SCD[37] and reduces sickling in the erythroid progeny of $CD34^+$ progenitors from SCD patients.[26,38] The BB305 and βAS3-FB vectors are currently in clinical development for gene therapy of SCD.

GENE THERAPY FOR β-THALASSEMIA

Beta thalassemia is caused by mutations that reduce (β^+) or abolish (β^0) the synthesis of β-globin chains,[39] resulting in ineffective erythropoiesis, intramedullary hemolysis, and hemolytic anemia. Clinical severity is variable and depends on the combination of different β^0 or β^+ mutations. The association with α-thalassemia or elevated HbF levels cause milder clinical phenotypes.[40,41] Patients with severe β-thalassemia present with

anemia, iron overload, hepatosplenomegaly, and skeletal abnormalities due to the BM expansion. Complications include cardiopathy, hepatic dysfunction, and endocrine disorders, which occur at various ages, depending on disease severity and adequacy of the transfusion and iron chelation treatment.[42] At present, the only cure for β-thalassemia is allogeneic HSC transplantation from HLA-identical donors, with implies high-dose chemotherapy and immunosuppression.[43] Transplantation with HSCs from a matched sibling donor results in >80% disease-free survival and 3–10% mortality on long-term follow-up,[2,44,45] but is limited by a donor availability of <30%.[46] Transplants from matched unrelated donors result in reduced disease-free survival and increased mortality,[47] while haploidentical transplantation carries unacceptably high risks. Chronic graft-versus-host disease, infections, and the toxicity associated with BM conditioning are common side effects of allogeneic HSC transplantation and affect both quality of life and survival. Gene therapy is theoretically available to all patients and, due to the autologous origin of the transplanted cells, is associated with reduced toxicity and treatment-related morbidity.

The traditional sources of stem cells for gene therapy are BM or peripheral blood mobilized $CD34^+$ HSPCs. Mobilization has essentially replaced BM harvest due to higher yield of $CD34^+$ cells, faster engraftment, enhanced immune reconstitution, and shorter hospitalization. The issue of HSC procurement is critical, since the minimal target dose of $CD34^+$ (usually $>5\times10^6$ cells/kg) is difficult to obtain by BM harvesting. Mobilization in the peripheral blood by administration of granulocyte-colony stimulating factor (G-CSF) provides adequate doses of $CD34^+$ HSPCs in most conditions but may be risky in β-thalassemia patients.[48] An alternative agent is Plerixafor (AMD3100; Mozobil™), a bicyclam molecule that antagonizes the binding of stromal cell-derived factor 1 to the chemokine CXC-receptor-4, causing mobilization of HSPCs in the peripheral circulation.[49] The use of Plerixafor in combination with G-CSF is currently approved in the United States and Europe for adult and pediatric populations.[50] Plerixafor, or G-CSF + Plerixafor, safely mobilizes adequate numbers of HSCs in β-thalassemia patients.[51–53] Plerixafor alone mobilizes HSCs with superior stem-cell characteristics, although with a somewhat lower yield compared to the association with G-CSF.[54] In the currently used protocols, mobilized $CD34^+$ cell populations are transduced by LVs and readministered to patients by intravenous or intrabone infusion. Adequate cell doses and clonal diversity of engrafted cells are necessary to reconstitute hematopoiesis rapidly and completely after transplantation, requiring high transduction efficiency and fully myeloablative conditioning regimens. The morbidity associated with conditioning is reduced in gene therapy compared to allogeneic HSC transplantation, since immune suppression is not required in an autologous setting.

The potential efficacy of HSC transduced with β-globin LVs in correcting the β-thalassemia phenotype was proven in murine models of the disease, starting from a pioneering study in 2000.[15,18,19,55–57] Interestingly, one of these studies showed an *in vivo* survival advantage for genetically corrected erythroblasts in a murine β-thalassemia model,[19] predicting that even suboptimal doses or transduced HSC may provide clinical benefit in patients. The potential genotoxicity of the currently used BB305 and GLOBE vectors was tested more recently in rigorous studies that fully supported clinical application.[21,58] Clinical development of gene therapy started in 2007 with the transplantation of HSCs transduced by the BGI LV expressing the β^{T87Q} globin in a patient affected by transfusion-dependent HbE/β-thalassemia.[12] The HbE trait mimics a mild β^+ allele and reduces the requirement for therapeutic β-globin synthesis. The patient received myeloablative busulfan conditioning, experienced a gradual increase in gene-marked cells up to 10–20%, and became transfusion independent with stable Hb levels of 8.5–9 g/dL 1 year after gene therapy. The mutant HbA, HbF, and HbE contributed almost equally to the therapeutic Hb levels.[12] Interestingly, the synthesis of therapeutic globin was largely accounted for by the expansion of a single stem/progenitor cell clone in which the LV integrated into the *HMGA2* proto-oncogene. The benign dominant clone persisted for almost 9 years, before declining to <10% of the circulating nucleated cells. The patient currently maintains stable levels of therapeutic Hb and requires only occasional transfusion (M. Cavazzana, unpublished observations). This pilot study proved the efficacy of gene therapy for β-thalassemia, but also indicated the requirement of an abundant, polyclonal population of transduced HSCs for significant and long-lasting clinical benefit. A clinical trial carried out in the United States using partially myeloablative conditioning showed minimal clinical benefit, confirming the requirement for full myeloablation for efficient engraftment of genetically corrected HSCs.[59]

Two subsequent clinical trials addressing transfusion-dependent β-thalassemia started in

2013 in the United States, Australia, Thailand, and France based on the use of the BB305 vector. At a median follow-up of 26 months, all but one of the 13 patients with a non β^0/β^0 thalassemia genotype had discontinued red cell transfusions and remained transfusion-independent with levels of total Hb of 8.2–13.7 g/dL, of which the therapeutic HbA^{T87Q} accounted for 3.4–10 g/dL. In nine patients with β^0/β^0 or severe β^+/β^+ (homozygous IVS1-110 mutation) genotypes, the median annualized transfusion volume decreased by 73%, and transfusions were discontinued in three patients. The treatment was well tolerated, with no severe adverse event related to gene transfer. Vector integration analysis showed polyclonal hematopoietic reconstitution and no evidence of vector-related clonal dominance.[14] These studies proved the safety and remarkable efficiency of LV-mediated gene therapy in reducing or eliminating the transfusion dependence in β-thalassemia patients.

Mobilization of HSPCs with Plerixafor + G-CSF was introduced in a Phase I/II clinical trial carried out in Italy addressing transfusion-dependent β-thalassemia. The study included three cohorts of adult (≥18 years; $n=3$), adolescent (8–17 years; $n=3$), and pediatric (3–7 years; $n=4$) subjects. Mobilized $CD34^+$ cells were transduced with the GLOBE vector and administered by intra-osseous injection in the posterior-superior iliac crests after myeloablative conditioning with treosulfan and thiotepa, with the rationale of favoring homing and engraftment.[60] As of March 2018, nine patients with different genotypes (β^0/β^0, β^+/β^+, and β^0/β^+) were treated, and the procedure was well tolerated, with no treatment-related adverse events. All tested patients showed multilineage cell engraftment and no evidence of clonal abnormality. Transfusion requirement was significantly reduced in the three adult patients, while three out of four evaluable pediatric participants discontinued transfusions and remained transfusion independent, with a follow-up of >12 months.[61]

Overall, the clinical trials (see list in Table 1) showed that clinical efficacy is directly correlated with gene transfer efficiency and the dose of genetically corrected HSCs and inversely correlated with the Hb synthesis requirement, where β^0 mutations are more difficult to correct than β^+ ones. Clinical efficacy also correlated with the average proviral copy number in transduced cells, and therefore with the overall output of therapeutic β-globin. In all trials, cell manufacturing appeared to be a critical factor, where continuous improvements in transduction conditions will play a major role in increasing efficacy and make gene therapy the treatment of choice for β-thalassemia patients.

GENE THERAPY FOR SCD

SCD is caused by a mutation (E6V) in the β-globin chain that induces polymerization of Hb tetramers upon deoxygenation. Hb polymers cause erythrocytes to adopt the characteristic sickle shape that reduces their flexibility in the capillary circulation, causing ischemia, multi-organ damage, severe pain, hemolytic anemia, and stroke, significantly shortening the patients' life-span.[7,62] Current treatments include periodical transfusion and administration of hydroxyurea, which moderately increases HbF synthesis.[62,63] Also for SCD, the only definitive treatment is allogeneic HSC transplantation from matched related donors, with a reported >90% disease-free survival over 6 years.[2] However, HSC transplantation is not frequently performed in SCD patients, given the toxicity and potential sterility associated with conditioning and the difficulty in finding suitable donors. As for β-thalassemia, gene therapy is a less toxic therapeutic option potentially available to all patients, though cell procurement and engraftment is even more challenging in the case of SCD.

Preclinical studies showed that LV-based gene therapy is potentially efficacious in reducing sickling. The vectors used in these studies contained the same combination of β-globin promoter and LCR elements used for β-thalassemia, and express anti-sickling globins such as the fetal γ-globin,[33,34] the β^{T87Q},[16] or the βAS3[26,37,38] mutants. As for β-

Table 1. *Active gene therapy clinical trials for β-thalassemia*

Country	*Sponsor*	*Gene*	*Vector*	*Conditioning*	*Enrolment*	*Identifier*	*Status*
United States	MSKCC	β-globin	TNS9.3.55	Busulfan: 8 mg/kg	Adults, 10 patients	NCT01639690	Active: 4 patients treated; not recruiting
France	Bluebird Bio	$\beta^{A\text{-}T87Q}$ globin	BB305	Busulfan: 12.8 mg/kg, pk-adjusted	Age 5–37 years, 7 patients	NCT02151526	Active: 4 patients treated; not recruiting
United States, Thailand, Australia	Bluebird Bio	$\beta^{A\text{-}T87Q}$ globin	BB305	Busulfan: 12.8 mg/kg	Adults ≤50 years, 23 patients	NCT02906202	Active: 18 patients treated; recruiting
Italy	Telethon Foundation	β-globin	GLOBE	Treosulfan 42 g/m^2 + Thiotepa 8 mg/kg	Age 3–64 years, 10 patients	NCT02453477	Active: 9 patients treated; recruiting

thalassemia, allogeneic HSC transplantation showed that stable mixed chimerism with donor HSC levels of 20–30% is sufficient to achieve significant hematologic and clinical improvement.[9–11] Combined with the evidence that the association of SCD and HPFH results in a milder phenotype, these data predict that engraftment of >20% of HSC producing RBCs with >30% anti-sickling Hb levels could improve symptoms and reduce transfusion requirement.

Clinical development of gene therapy for SCD started with a first patient treated in France, with transplantation of $CD34^+$ HSPCs transduced with the BB305 vector expressing the $\beta^{A\text{-}T87Q}$ globin at a dose of 5×10^6 cells/kg and an average vector copy number (VCN) of 1 after full myeloablative conditioning. The patient rapidly reconstituted a polyclonal hematopoiesis, achieved transfusion independence with a level of $\beta^{A\text{-}T87Q}$ globin of $\sim$50%, and experienced no treatment-related adverse event over a 2-year follow-up.[13] These results prompted the initiation of two clinical trials in France and the United States on severe SCD patients. However, the multicenter U.S. trial failed to reproduce the success of the first patient. The first seven patients treated received a median dose of 2×10^6 $CD34^+$ BM-derived HSPCs, with a median VCN of 0.6. A follow-up of 8–17 months showed a VCN in the peripheral blood of <0.12, HbA^{T87Q} levels of <2 g/dL, and an average HbA^{T87Q}/HbS ratio of <15%.[64] These results showed that mobilization and robust engraftment of transduced HSCs is more difficult to achieve in SCD than in β-thalassemia patients. Cell dose, transduction efficiency, and engraftment are apparently limiting factors in achieving the minimal HSC chimerism and synthesis of anti-sickling Hb in erythroblasts predicted to provide clinical benefit.

Harvesting $CD34^+$ cells is a risky procedure in SCD patients, which often fails to provide an adequate cell dose. In this regard, the BM microenvironment appears to be a critical factor. Capillary occlusion caused by sickling leads to ischemia, inflammation, and oxidative stress,[65] which compromise the quality of the HSC niche and impact on both harvest and re-engraftments of HSCs. Attempts to mobilize HSCs by low doses of G-CSF caused severe adverse events and at least one fatality, and was therefore abandoned.[66] A Plerixafor-only mobilization trial recently completed in France proved the safety of the drug in an SCD context and provided an estimate of the yield of $CD34^+$ HSPCs in both adult and juvenile patients.[67] Plerixafor mobilization has been recently implemented in the ongoing gene therapy clinical trials (see list in Table 2). A better cell quality and dose, together with ongoing innovation in cell manufacturing to increase transduction efficiency, should rapidly improve the clinical outcome of gene therapy for SCD.

GENE THERAPY FOR HEMOGLOBINOPATHIES: FUTURE DIRECTIONS

A limitation of the *ex vivo* gene therapy approach for hemoglobinopathies is the complex, difficult, and very expensive cell manufacturing step. Administering a globin vector directly *in vivo* would make gene therapy easier to perform and more feasible in less favored country, where cost and complexity represent formidable barrier to patient access. Approaches based on the use of capsid-modified adenovirus vectors and the integration-promoting machinery of the *Sleeping Beauty* transposon have been recently published[68] and may represent a promising alternative to LVs to integrate a β-globin expression cassette in repopulating stem cells directly into the BM.

Gene editing has recently emerged as a potential alternative to vector-mediated gene addition for gene therapy of β-hemoglobinopathies. Editing the β^S mutation by homology-directed DNA repair (HDR) may be achieved by using zinc-finger nucleases or CRISPR/Cas9 to cleave the β^S locus and viral genomes or single-stranded oligonucleotides as HDR donor templates. Correction of the β^S mutation can be obtained in $CD34^+$ HSPCs with remarkable efficiency, achieving potentially therapeutic

Table 2. *Active gene therapy clinical trials for sickle-cell disease*

Country	*Sponsor*	*Gene*	*Vector*	*Conditioning (TD)*	*Enrolment*	*Identifier*	*Status*
France	Bluebird Bio	$\beta^{A\text{-}T87Q}$ globin	BB305	Busulfan: 12.8 mg/kg, pk-adjusted	Age 5–37 years, 7 patients	NCT02151526	Active: 3 patients treated; not recruiting
United States	Bluebird Bio	$\beta^{A\text{-}T87Q}$ globin	BB305	Busulfan: 12.8 mg/kg	Adults, 29 patients	NCT02140554	Active: 9 patients treated
United States	UCLA	βAS3 globin	βAS3-FB	Busulfan: 12.8 mg/kg, pk-adjusted	Adults, 6 patients	NCT02247843	Active: 1 patient treated; recruiting
United States, Jamaica	Cincinnati Children's Hospital	γ-globin	mLAR$\beta\Delta\gamma$V5	Melphalan: 140 mg/m^2	Age 18–35 years, 10 patients	NCT02186418	Active: recruiting
United States	Boston Children's Hospital	BCL11A shRNA^{mir}	LCR-shRNA^{mir}	Busulfan: 12.8 mg/kg, pk-adjusted	Age 3–40 years, 7 patients	NCT03282656	Active, 1 patient treated; recruiting

HbA/HbS ratios in the erythroid progeny of edited cells differentiated *in vitro* or *in vivo* in NOD SCID gamma mice.[69–72]

An alternative strategy aims at reactivating HbF synthesis in adult erythroblasts. A first approach is based on downregulating BCL11A, a transcription factor involved in silencing γ-globin expression in adult erythroblasts.[73,74] Downregulating BCL11A by restricted expression of an shRNA in the erythroid compartment after delivery to HSCs by a GLOBE-like vector is a potentially efficacious strategy[75] that recently entered clinical investigation (see Table 2). Fine modulation of the shRNA is, however, necessary, since BCL11A is essential for HSC and lymphoid cell development[76] and may be required for RBC enucleation.[77] Alternatively, BCL11A can be down-modulated by deleting the erythroid-specific enhancers controlling its erythrocyte-restricted expression by CRISPR/Cas9-mediated genome editing.[78,79] A second approach is based on recreating genomic deletions or mutations in the β-globin locus associated with HPFH,[7,32] leading to increased γ-globin expression[80–82] although with subtherapeutic efficiency compared to LV-mediated gene replacement. This strategy is less complex than editing the β^S mutation, since it relies on non-homologous end joining, the dominant DNA repair pathway in HSPCs,[69,83] and does not require delivering a DNA template. A more creative approach is based on the use of ZF protein coupled to an effector domain, inducing a loop between the LCR and the γ-globin promoters, which reactivates γ-globin synthesis at the expenses of β^S-globin without requiring DNA cleavage.[84]

Editing the genome holds great promises for gene therapy of hemoglobinopathies and may offer advantages with respect to LV-mediated gene replacement in terms of complexity and cost of manufacturing genetically modified HSCs. However, this technology has still to prove its efficacy and biosafety profile in a real-life context. Off-target effects and unrepaired double-stranded cuts may represent significant risk factors, not necessarily inferior to the potential genotoxic effects of LV integration in the human genome. Given the absence of reliable preclinical models to analyze editing efficiency in long-term repopulating HSCs and any associated genotoxicity, the efficacy and safety of gene editing may eventually be proven only in clinical trials.

AUTHOR DISCLOSURE

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REFERENCES

1. Modell B and Darlison M. Global epidemiology of hemoglobin disorders and derived service indicators. Bull World Health Organ 2008;86:480–487.
2. Locatelli F, Kabbara N, Ruggeri A, et al. Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling. Blood 2013;122:1072–1078.
3. Naldini L. Gene therapy returns to centre stage. Nature 2015;526:351–360.
4. Biasco L, Baricordi C, Aiuti A. Retroviral integrations in gene therapy trials. Mol Ther 2012;20:709–716.
5. Biasco L, Pellin D, Scala S, et al. *In vivo* tracking of human hematopoiesis reveals patterns of clonal dynamics during early and steady-state reconstitution phases. Cell Stem Cell 2016;19:107–119.
6. Poletti V, Mavilio F. Interactions between retroviruses and the host cell genome. Mol Ther Methods Clin Dev 2018;8:31–41.
7. Stamatoyannopoulos G. The Molecular Basis of Blood Diseases, 3rd ed. Philadelphia, PA: W.B. Saunders Co., 2001.
8. Wilber A, Nienhuis AW, Persons DA. Transcriptional regulation of fetal to adult hemoglobin switching: new therapeutic opportunities. Blood 2011;117:3945–3953.
9. Andreani M, Testi M, Gaziev J, et al. Quantitatively different red cell/nucleated cell chimerism in patients with long-term, persistent hematopoietic mixed chimerism after bone marrow transplantation for thalassemia major or sickle cell disease. Haematologica 2011;96:128–133.
10. Walters MC, Patience M, Leisenring W, et al. Stable mixed hematopoietic chimerism after bone marrow transplantation for sickle cell anemia. Biol Blood Marrow Transplant 2001;7:665–673.
11. Wu CJ, Gladwin M, Tisdale J, et al. Mixed haematopoietic chimerism for sickle cell disease prevents intravascular haemolysis. Br J Haematol 2007;139:504–507.
12. Cavazzana-Calvo M, Payen E, Negre O, et al. Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. Nature 2010;467:318–322.
13. Ribeil JA, Hacein-Bey-Abina S, Payen E, et al. Gene therapy in a patient with sickle cell disease. New Engl J Med 2017;376:848–855.
14. Thompson AA, Walters MC, Kwiatkowski J, et al. Gene therapy in patients with transfusion-dependent beta-thalassemia. New Engl J Med 2018;378:1479–1493.
15. May C, Rivella S, Callegari J, et al. Therapeutic haemoglobin synthesis in beta-thalassaemic mice expressing lentivirus-encoded human beta-globin. Nature 2000;406:82–86.
16. Pawliuk R, Westerman KA, Fabry ME, et al. Correction of sickle cell disease in transgenic mouse models by gene therapy. Science 2001;294:2368–2371.
17. May C, Rivella S, Chadburn A, et al. Successful treatment of murine beta-thalassemia intermedia by transfer of the human beta-globin gene. Blood 2002;99:1902–1908.
18. Rivella S, May C, Chadburn A, et al. A novel murine model of Cooley anemia and its rescue by lentiviral-mediated human beta-globin gene transfer. Blood 2003;101:2932–2939.
19. Miccio A, Cesari R, Lotti F, 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;105:10547–10552.
20. Roselli EA, Mezzadra R, Frittoli MC, et al. Correction of beta-thalassemia major by gene transfer in haematopoietic progenitors of pediatric patients. EMBO Mol Med 2010;2:315–328.

21. Negre O, Bartholomae C, Beuzard Y, 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.

22. Arumugam PI, Scholes J, Perelman N, et al. Improved human beta-globin expression from self-inactivating lentiviral vectors carrying the chicken hypersensitive site-4 (cHS4) insulator element. Mol Ther 2007;15:1863–1871.

23. Lisowski L, Sadelain M. Locus control region elements HS1 and HS4 enhance the therapeutic efficacy of globin gene transfer in beta-thalassemic mice. Blood 2007;110:4175–4178.

24. Emery DW, Yannaki E, Tubb J, et al. A chromatin insulator protects retrovirus vectors from chromosomal position effects. Proc Natl Acad Sci U S A 2000;97:9150–9155.

25. Ramezani A, Hawley TS, Hawley RG. Combinatorial incorporation of enhancer-blocking components of the chicken beta-globin 5′HS4 and human T-cell receptor alpha/delta BEAD-1 insulators in self-inactivating retroviral vectors reduces their genotoxic potential. Stem Cells 2008;26:3257–3266.

26. Romero Z, Urbinati F, Geiger S, et al. β-globin gene transfer to human bone marrow for sickle cell disease. J Clin Invest 2013;123:3317–3330.

27. Negre O, Eggimann AV, Beuzard Y, et al. Gene therapy of the beta-hemoglobinopathies by lentiviral transfer of the beta(A(T87Q))-globin gene. Hum Gene Ther 2016;27:148–165.

28. Miccio A, Poletti V, Tiboni F, et al. The GATA1-HS2 enhancer allows persistent and position-independent expression of a beta-globin transgene. PLoS One 2011;6:e27955.

29. Breda L, Casu C, Gardenghi S, et al. Therapeutic hemoglobin levels after gene transfer in beta-thalassemia mice and in hematopoietic cells of beta-thalassemia and sickle cells disease patients. PLoS One 2012;7:e32345.

30. Romero Z, Campo-Fernandez B, Wherley J, et al. The human ankyrin 1 promoter insulator sustains gene expression in a beta-globin lentiviral vector in hematopoietic stem cells. Mol Ther Methods Clin Dev 2015;2:15012.

31. Akinsheye I, Alsultan A, Solovieff N, et al. Fetal hemoglobin in sickle cell anemia. Blood 2011; 118:19–27.

32. Steinberg MH, Chui DH, Dover GJ, et al. Fetal hemoglobin in sickle cell anemia: a glass half full? Blood 2014;123:481–485.

33. Pestina TI, Hargrove PW, Jay D, et al. Correction of murine sickle cell disease using gamma-globin lentiviral vectors to mediate high-level expression of fetal hemoglobin. Mol Ther 2009;17:245–252.

34. Perumbeti A, Higashimoto T, Urbinati F, et al. A novel human gamma-globin gene vector for genetic correction of sickle cell anemia in a humanized sickle mouse model: critical determinants for successful correction. Blood 2009; 114:1174–1185.

35. McCune SL, Reilly MP, Chomo MJ, et al. Recombinant human hemoglobins designed for gene therapy of sickle cell disease. Proc Natl Acad Sci U S A 1994;91:9852–9856.

36. Levasseur DN, Ryan TM, Reilly MP, et al. A recombinant human hemoglobin with anti-sickling properties greater than fetal hemoglobin. J Biol Chem 2004;279:27518–27524.

37. Levasseur DN, Ryan TM, Pawlik KM, et al. Correction of a mouse model of sickle cell disease: lentiviral/antisickling beta-globin gene transduction of unmobilized, purified hematopoietic stem cells. Blood 2003;102:4312–4319.

38. Urbinati F, Hargrove PW, Geiger S, et al. Potentially therapeutic levels of anti-sickling globin gene expression following lentivirus-mediated gene transfer in sickle cell disease bone marrow CD34+ cells. Exp Hematol 2015; 43:346–351.

39. Higgs DR, Engel JD, Stamatoyannopoulos G. Thalassaemia. Lancet 2012;379:373–383.

40. Chang YP, Littera R, Garau R, et al. The role of heterocellular hereditary persistence of fetal haemoglobin in beta(0)-thalassaemia intermedia. Br J Haematol 2001;114:899–906.

41. Panigrahi I, Agarwal S. Genetic determinants of phenotype in beta-thalassemia. Hematology 2008; 13:247–252.

42. Rund D, Rachmilewitz E. Beta-thalassemia. New Engl J Med 2005;353:1135–1146.

43. Angelucci E. Hematopoietic stem cell transplantation in thalassemia. Hematology Am Soc Hematol Educ Program 2010;2010:456–462.

44. Lucarelli G, Gaziev J. Advances in the allogeneic transplantation for thalassemia. Blood Rev 2008; 22:53–63.

45. Isgro A, Gaziev J, Sodani P, et al. Progress in hematopoietic stem cell transplantation as allogeneic cellular gene therapy in thalassemia. Ann N Y Acad Sci 2010;1202:149–154.

46. Angelucci E, Matthes-Martin S, Baronciani D, et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. Haematologica 2014;99:811–820.

47. La Nasa G, Caocci G, Argiolu F, et al. Unrelated donor stem cell transplantation in adult patients with thalassemia. Bone Marrow Transplant 2005; 36:971–975.

48. Taher AT, Otrock ZK, Uthman I, et al. Thalassemia and hypercoagulability. Blood Rev 2008;22: 283–292.

49. De Clercq E. The bicyclam AMD3100 story. Nat Rev Drug Discov 2003;2:581–587.

50. DiPersio JF, Stadtmauer EA, Nademanee A, et al. Plerixafor and G-CSF versus placebo and G-CSF to mobilize hematopoietic stem cells for autologous stem cell transplantation in patients with multiple myeloma. Blood 2009;113:5720–5726.

51. Yannaki E, Karponi G, Zervou F, et al. Hematopoietic stem cell mobilization for gene therapy: superior mobilization by the combination of granulocyte-colony stimulating factor plus Plerixafor in patients with beta-thalassemia major. Hum Gene Ther 2013;24:852–860.

52. Yannaki E, Papayannopoulou T, Jonlin E, et al. Hematopoietic stem cell mobilization for gene therapy of adult patients with severe beta-thalassemia: results of clinical trials using G-CSF or Plerixafor in splenectomized and nonsplenectomized subjects. Mol Ther 2012;20:230–238.

53. Karponi G, Psatha N, Lederer CW, et al. Plerixafor + G-CSF-mobilized CD34+ cells represent an optimal graft source for thalassemia gene therapy. Blood 2015;126:616–619.

54. Lidonnici MR, Aprile A, Frittoli MC, et al. Plerixafor and G-CSF combination mobilizes hematopoietic stem and progenitors cells with a distinct transcriptional profile and a reduced *in vivo* homing capacity compared to Plerixafor alone. Haematologica 2017;102:e120–e124.

55. Imren S, Payen E, Westerman KA, 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;99:14380–14385.

56. Persons DA, Allay ER, Sawai N, et al. Successful treatment of murine beta-thalassemia using *in vivo* selection of genetically-modified, drug-resistant hematopoietic stem cells. Blood 2003;102:506–516.

57. Hanawa H, Hargrove PW, Kepes S, et al. Extended beta-globin locus control region elements promote consistent therapeutic expression of a gamma-globin lentiviral vector in murine beta-thalassemia. Blood 2004;104:2281–2290.

58. Lidonnici MR, Paleari Y, Tiboni F, et al. Multiple integrated non-clinical studies predict safety of lentiviral mediated gene therapy for beta thalassemia. Mol Ther Methods Clin Dev 2018. (in press).

59. Mansilla-Soto J, Riviere I, Boulad F, et al. Cell and gene therapy for the beta-thalassemias: advances and prospects. Hum Gene Ther 2016;27:295–304.

60. Bernardo M, Piras E, Vacca 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;120:473–126.

61. Scaramuzza S, Marktel S, Cicalese MP et al. Lentiviral hematopoietic stem cells gene therapy for beta-thalassemia: update from the phase I/II TIGET BTHAL trial. ASGCT 21st Annual Meeting. Mol Ther 2018;26:S1.

62. Madigan C, Malik P. Pathophysiology and therapy for haemoglobinopathies. Part I: sickle cell disease. Expert Rev Mol Med 2006;8:1–23.

63. Platt OS. Hydroxyurea for the treatment of sickle cell anemia. New Engl J Med 2008;358:1362–1369.

64. Kanter J, Walters MC, Hsieh MM, et al. Interim results from a Phase 1/2 clinical study of lentiglobin

gene therapy for severe sickle cell disease. Blood 2016;128.

65. Zhang D, Xu C, Manwani D, et al. Neutrophils, platelets, and inflammatory pathways at the nexus of sickle cell disease pathophysiology. Blood 2016;127:801–809.
66. Fitzhugh CD, Hsieh MM, Bolan CD, et al. Granulocyte colony-stimulating factor (G-CSF) administration in individuals with sickle cell disease: time for a moratorium? Cytotherapy 2009;11:464–471.
67. Lagresle-Peyrou C, Lefrere F, Magrin E, et al. Plerixafor enables safe, rapid, efficient mobilization of hematopoietic stem cells in sickle cell disease patients after exchange transfusion. Haematologica 2018;103:778–786.
68. Li C, Psatha N, Wang H, et al. Integrating HDAd5/35++ vectors as a new platform for HSC gene therapy of hemoglobinopathies. Mol Ther Methods Clin Dev 2018;9:142–152.
69. Hoban MD, Cost GJ, Mendel MC, et al. Correction of the sickle cell disease mutation in human hematopoietic stem/progenitor cells. Blood 2015; 125:2597–2604.
70. Hoban MD, Lumaquin D, Kuo CY, et al. CRISPR/Cas9-mediated correction of the sickle mutation in human CD34+ cells. Mol Ther 2016;24:1561–1569.
71. DeWitt MA, Magis W, Bray NL, et al. Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells. Sci Transl Med 2016;8:360ra134.
72. Dever DP, Bak RO, Reinisch A, et al. CRISPR/Cas9 beta-globin gene targeting in human haematopoietic stem cells. Nature 2016;539:384–389.
73. Bauer DE, Orkin SH. Hemoglobin switching's surprise: the versatile transcription factor BCL11A is a master repressor of fetal hemoglobin. Curr Opin Genet Dev 2015;33:62–70.
74. Smith EC, Orkin SH. Hemoglobin genetics: recent contributions of GWAS and gene editing. Hum Mol Genet 2016;25:R99–R105.
75. Brendel C, Guda S, Renella R, et al. Lineage-specific BCL11A knockdown circumvents toxicities and reverses sickle phenotype. J Clin Invest 2016; 126:3868–3878.
76. Tsang JC, Yu Y, Burke S, et al. Single-cell transcriptomic reconstruction reveals cell cycle and multi-lineage differentiation defects in Bcl11a-deficient hematopoietic stem cells. Genome Biol 2015;16:178.
77. Chang K-H, Smith SE, Sullivan T, et al. Long-term engraftment and fetal globin induction upon *BCL11A* gene editing in bone-marrow-derived $CD34^+$ hematopoietic stem and progenitor cells. Mol Ther Methods Clin Dev 2017;4:137–148.
78. Canver MC, Smith EC, Sher F, et al. BCL11A enhancer dissection by Cas9-mediated *in situ* saturating mutagenesis. Nature 2015;527:192–197.
79. Vierstra J, Reik A, Chang K-H, et al. Functional footprinting of regulatory DNA. Nat Methods 2015;12:927–930.
80. Traxler EA, Yao Y, Wang Y-D, et al. A genome-editing strategy to treat [beta]-hemoglobinopathies that recapitulates a mutation associated with a benign genetic condition. Nat Med 2016;22: 987–990.
81. Ye L, Wang J, Tan Y, et al. Genome editing using CRISPR-Cas9 to create the HPFH genotype in HSPCs: an approach for treating sickle cell disease and beta-thalassemia. Proc Natl Acade Sci U S A 2016;113:10661–10665.
82. Antoniani C, Meneghini V, Lattanzi A, et al. Induction of fetal hemoglobin synthesis by CRISPR/Cas9-mediated editing of the human beta-globin locus. Blood 2018;131:1960–1973.
83. Genovese P, Schiroli G, Escobar G, et al. Targeted genome editing in human repopulating haematopoietic stem cells. Nature 2014;510:235–240.
84. Breda L, Motta I, Lourenco S, et al. Forced chromatin looping raises fetal hemoglobin in adult sickle cells to higher levels than pharmacologic inducers. Blood 2016;128:1139–1143.

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