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INVITED REVIEW ARTICLE

Gene therapy of hemoglobinopathies: progress and future challenges

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Abstract

Recently, gene therapy clinical trials have been successfully applied to hemoglobinopathies, such as sickle cell disease (SCD) and β-thalassemia. Among the great discoveries that led to the design of genetic approaches to cure these disorders is the discovery of the β-globin locus control region and several associated transcription factors, which determine hemoglobin switching as well as high-level, erythroid-specific expression of genes at the ß-globin locus. Moreover, increasing evidence shows that lentiviral vectors are efficient tools to insert large DNA elements into nondividing hematopoietic stem cells, showing reassuring safe integration profiles. Alternatively, genome editing could restore expression of fetal hemoglobin or target specific mutations to restore expression of the wild-type β-globin gene. The most recent clinical trials for β-thalassemia and SCD are showing promising outcomes: patients were able to discontinue transfusions or had reduced transfusion requirements. However, toxic myeloablation and the high cost of current *ex vivo* hematopoietic stem cell gene therapy platforms represent a barrier to a widespread application of these approaches. In this review, we summarize these gene therapy strategies and ongoing clinical trials. Finally, we discuss possible strategies to improve outcomes, reduce myeloablative regimens and future challenges to reduce the cost of gene therapy platform.

Introduction

Hemoglobin (Hb), the oxygen-carrying molecule, contains two identical α- and β-globin chains linked to a heme group (1). ß-globinopathies are inherited disorders characterized by mutations that decrease the synthesis of the ß-globin chain, as in ß-thalassemia, or lead to a mutated ß-globin variant, as in sickle cell disease (SCD) (β^S), which causes hemoglobin polymerization

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and red blood cell sickling (2). Several chronic treatments are available, but the main curative option for SCD and ß-thalassemia is allogenic hematopoietic stem cell transplantation (HSCT) from HLA-genoidentical donors. This, however, is available to only less than 20% of patients and associated with potential morbidity and mortality especially when realized from a donor other than an HLA-identical sibling and in adults affected by severe organ damage (3,4). Haploidentical transplants also are potentially curative (5–9). In this case, the number of matched HLA unrelated donors is higher (>90%), but graft rejection still represents the major issue (5–9) (50% to 60%). Gene therapy, by use of genetically modified autologous hematopoietic stem cell (HSCs) and autologous-HSCT, represents a potential alternative option.

Over the past several decades, expression of curative genes using a housekeeping promoter into self-inactivating lentiviral vectors (SIN-LVs) have been successfully utilized to correct diseases such as adenosine deaminase-deficient severe combined immunodeficiency (ADA-SCID), Wiskott–Aldrich syndrome, X-linked SCID and congenital metabolic diseases (e.g. adrenoleukodystrophy or metachromatic leukodystrophy), to name a few (10,11). These trials provided strong evidence of clinical long-term efficacy in the absence of genotoxicity (12).

However, gene therapy for hemoglobinopathies faced additional challenges. High-level and erythroid-restricted transcription of a curative globin gene requires the interaction of the globin promoters with the locus control region (LCR) (13,14). Although the combination of a full LCR and an adult β-globin gene, including its introns and 3′ untranslated regions, has showed satisfactory levels of gene expression (15) , the full LCR is too large to fit into a lentiviral vector (LV) construct (16). Therefore, modified LVs containing shortened but still large transgene expression cassettes have been developed and successfully tested in preclinical studies for hemoglobinopathies. Recent clinical trials have shown remarkable safety and promising clinical efficacy (17–19).

However, the high cost of current *ex vivo* HSC gene therapy is a barrier to a widespread application to treat a variety of diseases. The first gene therapy approved in the Western world is set to go on sale in Germany and USA at a cost close to $1 million per treatment (20–22). For bluebird bio product LentiGlobin, for patients with transfusion-dependent beta-thalassemia, while exact pricing details are not yet available, the gene therapy is expected to carry a six-figure price tag. For this last therapy, patients require intensive care due to the myeloablative conditioning regimen and a large amount of virus supernatant to transduce efficiently HSC. These are the main causes for the high cost of this approach.

In this review, we will summarize the globin gene expression system, the clinical studies that have been open worldwide and the pre-clinical studies for improving outcomes. Finally, we will discuss recent work to address the financial barriers.

Background of gene therapy: control of β-like globin gene expression

In humans, two gene clusters direct the synthesis of Hb: the α locus, which contains the embryonic ζ gene and two adult α genes; and the β locus, which consists of ε, $^{G}\gamma$, $^{A}\gamma$, δ and β genes. Two globin gene switches occur during development: the embryonic to fetal globin switch and the fetal to adult switch. The switches from ε to γ and from γ to β globin gene expression are controlled exclusively at the transcription level. In addition to the individual regulation of each globin gene by transcription factors, the transcription of the entire cluster is subjected to a shared control region indicated as LCR, lying 40–60 kb upstream of the β-globin gene. The LCR interacts with the promoter regions of the active genes by forming stem-loop structures (23). The LCR is composed of multiple hypersensitivity sites (HS1, HS2, HS3, HS4 and HS5) with high affinity for transcription factors. Initial attempts to develop integrating γ-retroviral vectors for β-globin gene transfer revealed challenges to attain efficient, high-level erythroid-specific expression because of lack of the LCR in the vector (24–27). Viral long terminal repeat (LTR)-driven transgene expression of β-globin resulted in little or no β-globin expression. However, the development of an LV that included a β-globin cassette with a portion of the LCR, showed major breakthroughs (28,29). This new generation of vectors, expressing β-like globin (wild-type β-globin or anti-sickling β-like globin, such as fetal γ-globin) at much higher levels proved efficacious in correcting two mouse models of β-thalassemia (28,30,31) and SCD (29), paving the way to clinical trials.

Induction of endogenous fetal hemoglobin (HbF, $\alpha_2\gamma_2$) is another strategy for gene therapy in SCD and β-thalassemia. This approach has the advantage to lead to therapeutic expression of the endogenous γ-globin genes under the control of the LCR and, in the case of SCD, reduction of the β^S-globin synthesis. Decades ago, it was established that β-thalassemia and SCD patients experience less severe symptoms when HbF is elevated due to the condition known as hereditary persistence of fetal hemoglobin (HPFH) (32–35). In β-thalassemia, elevated γ-globin levels compensate for the β-globin deficiency, improving the imbalance in the ratio of α to β-like globins and ameliorating anemia. The mechanisms of protective activity by fetal globins in SCD is that γ-globin chains are incorporated into mixed hemoglobin tetramers that do not participate in polymer formation, thus inhibiting sickling and leading to a less severe clinical phenotype. In SCD, HbF levels greater than 20% ameliorates severity of disease symptoms and improves patient survival. The B cell CLL/lymphoma 11A (BCL11A) protein is a major modulator of HbF expression (36,37), and genetic variation in the erythroid enhancer of *BCL11A* affects its expression and, consequently, HbF levels (38). Patients with *BCL11A* haploinsufficiency have increased levels of HbF at levels likely to be therapeutic for patients with β-hemoglobinopathies (39). The challenge has been to suppress expression of BCL11A only in erythroid cells, since disruption *BCL11A* has critical physiological functions beyond globin control. *BCL11A* plays important roles in HSCs self-renewal, B-lymphocyte maturation and development of the central nervous system (40–43). To this end, multiple approaches are being explored to genetically induce HbF expression. Initial studies showed that knockdown of *BCL11A* using an LV shRNA/miRNA approach causes induction of HbF in human erythroid cells (44,45). Since narrow enhancer sequences are responsible for expression of *BCL11A* in erythroid cells, single cleavage by a nuclease, whether it could be zinc-finger nucleases (ZFN), transcription activator like effector nucleases (TALENs) or CRISPR-Cas9, may be sufficient to disrupt *BCL11A* expression in red cells resulting in elevated HbF level (46,47). Several groups succeeded in the functional correction of thalassemic and SCD $CD34^+$ cells *in vitro* and *in vivo* by either ZFN or CRISPR-Cas9 inactivation of the *BCL11A*-erythroid enhancer (48). These reports clearly showed that novel and effective genome editing strategies for suppressing *BCL11A* gene expression are promising treatment options for hemoglobinopathies (49). In addition, editing approaches could be theoretically safer compared to LVs integrating semi-randomly in the genome (50,51). Clinical trials based on this approach are in progress (Table 1).

Table 1. Main gene therapy trials for ß-hemoglobinopathies

Clinical trial #	Phase	Disease	Site	LV/Nuclease	Sponsor
NCT02151526 (HGB205)	1/2	ß-thal and SCD	France	BB305 LV	bluebird bio
NCT01745120 (HGB204)	1/2	ß-thal	USA, Australia, Thailand	BB305 LV	bluebird bio
NCT02140554 (HGB206)	1/2	SCD	USA	BB305 LV	bluebird bio
NCT02906202 (HGB207 and HGB212)	3	ß-thal	USA, France, Germany, Greece, Italy, Thailand, UK,	TNS9.3.55 LV	bluebird bio
NCT01639690	1	ß-thal	USA	TNS9.3.55 LV	Memorial Sloan Kettering Cancer Center
NCT02453477	1/2	ß-thal	Italy	GLOBE LV	IRCCS San Raffaele
NCT02186418	1/2	SCD	USA, Jamaica	sGbG LV	Children's Hospital Medical Center,
NCT02247843	1	SCD	USA	βAS3-FB LV	University of California Children's Hospital
NCT03282656	1	ß-thal	USA	BCH_BB-LCRshRNA (miR) LV	David Williams Boston Children's Hospital
NCT03432364	1/2	ß-thal	USA	ZFN (BCL11A enhancer)	Sangamo Therapeutics and Bioverativ Therapeutics Inc.
NCT03655678	1/2	ß-thal	Germany, UK	CRISPR/Cas9 (BCL11A enhancer)	Vertex Pharmaceuticals Incorporated and CRISPR Therapeutics
NCT03745287	1/2	SCD	USA	CRISPR/Cas9 (BCL11A enhancer)	Vertex Pharmaceuticals Incorporated and CRISPR Therapeutics

LV, lentiviral vector; ß-thal, ß-thalassemia; SCD, sickle cell disease.

Clinical trials for β-thalassemia: β0/0 patients, a challenging phenotype

Initial observations from the clinical trials of the LentiGlobin BB305 vector (NCT02151526 and NCT01745120, BB305 vector; Table 1) showed that 11 out of 13 patients who had a non-β0/0 genotype were able to discontinue red cell transfusions (19,52). However, treatment for the severe phenotype of β-thalassemia (β0/0) has been proved more challenging. We previously reported that β0/0 patients required higher vector copy number (VCN) to reach therapeutic levels of Hb after transduction with an LV carrying the β-globin gene and indicated as AnkT9W, whereas β0/+ and β+/+ benefitted from the expression of the endogenous HbA and reached curative levels at lower VCN (53). In the same trials of LentiGlobin BB305, five out of nine patients with a β0/0 genotype or two copies of IVS1-110 mutation, which is a non-β0/0 form with a severe disease phenotype, still require transfusions (19). These results indicate that, while non-β0/0 genotypes might require relatively low levels of genomic integrations of the vector to reach transfusion independence, β0/0 or IVS1-110 genotypes require higher number of vector copies to achieve complete curative endpoints.

In the trial sponsored by IRCCS San Raffaele for β-thalassemia (NCT02453477, Table 1), transfusion requirement was reduced in the three adult patients with severe β0/IVS110 genotype, and three of the four pediatric patients (including one with an IVS110/IVS110 genotype and one with a β0/0 genotype) became transfusion-independent. In the transfusion-independent patients, full correction of the anemia was obtained only in the presence of a high VCN (51).

Similarly, preliminary data of the NCT02906202/HGB212 trial (Table 1) indicated that higher levels of transduction were able to increase the Hb levels to 13 g/dL in one patient and to 10 g/dL in other two patients with the phenotype β0/0 (54). While these data support the use of gene therapy approaches to cure hemoglobinopathies, the potential concern is that high VCN may also lead to increased risk of insertional mutagenesis. Furthermore, the requirement for high VCN in a pancellular fashion may also preclude the use of these vectors for reduced myeloablative regimens (minitransplants) that would allow for reduced toxic conditioning, but would also reduce the number of genetically modified engrafted HSCs. To this end, modification of the LV vector to express higher levels of hemoglobin might be required. Novel vectors could also potentially carry modified and more functional β-globin and simultaneously suppress expression of *BCL11A* in the erythroid lineage. In this setting, where cells express high enough levels of therapeutic globins, mixed chimerism might be acceptable.

Clinical trials for sickle cell anemia

The results obtained in β-thalassemia encouraged attempts to extend gene therapy to SCD patients. A first patient treated in France with the BB305 LV rapidly achieved a level of therapeutic anti-sickling globin (HbA^{T87Q}) of ~50% with clinical and biological parameters typical of SCD carriers (18). Of note, only one out of the two other patients treated in the same clinical trial had benefitted from the treatment, producing 40% of anti-sickling Hb (HbA^{T87Q}+HbF).

A subsequent multicenter clinical trial carried out in the US with the same vector demonstrated the importance of several parameters. After the first seven treated patients, the protocol was modified to introduce a red blood transfusion regimen before the HSC harvest and a real-time pharmocokinetic study of busulphan during the myeloablation. Moreover, the new protocol included modifications in the number of the transplanted $CD34^+$ hematopoietic/stem progenitor cells and transduction protocol [NCT02140554/HGB206 (54,55), Table 1]. Of note, 6 months after gene therapy, the last group of patients showed a median HbS levels inferior to 50% and hemolysis parameters decreased to normal levels (Kanter J et al EHA 2019). Based on these results, it is highly recommended to infuse an average number of 7×10^6 $CD34^+$ cells/kg with a VCN ≥2 for BB305 LV. Furthermore, to obtain a high cell number, HSCs from SCD patients have to be mobilized in the peripheral blood by plerixafor under controlled conditions (56).

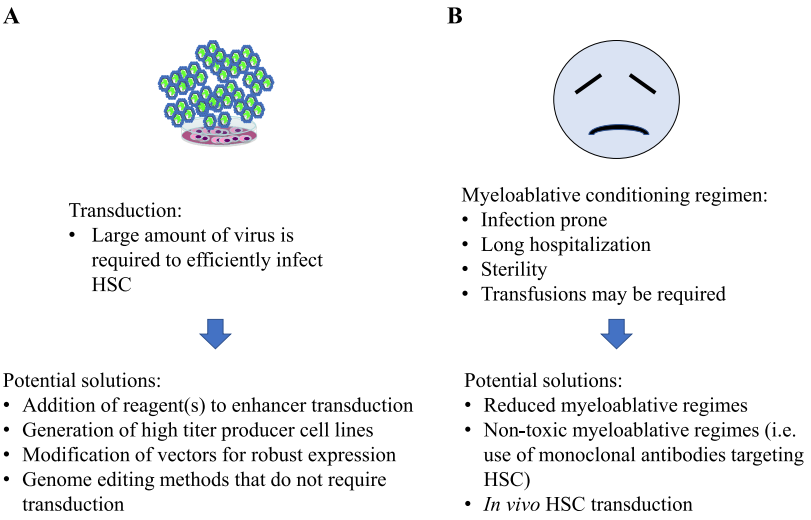

Figure 1. Future plans for the solution of high cost LV transduction and to reduce the side effects of myeloablation.

In the USA, two other Phase I/II trials for SCD have been initiated; Malik et al. (57) treated two $\beta^S/\beta 0$ patients after a reduced intensity conditioning regimen using an LV expressing a modified γ-globin transgene (NCT02186418, Table 1); the LV-induced HbF accounted for ∼20% to 30% of the total Hb and the patients displayed a significant improvement in clinical and biological parameters; the favorable $\beta^S/\beta 0$ genetic background, which is associated with reduced β^S intracellular concentration, may have contributed to the favorable outcome. In the second clinical trial, a vector containing a β-globin transgene with three anti-sickling mutations is being utilized, but to date no results are available (NCT02247843, Table 1).

Besides gene therapy by gene addition of an anti-sickling globin gene, another approach to correct the SCD phenotype aims at inducing endogenous HbF. In a current open gene therapy trial (NCT03282656, Table 1), the HbF induction strategy is based on introduction of shRNA targeting BCL11A suppression in erythroid cells derived from HSCs for the purpose of increasing γ-globin and reducing mutant β^S globin expression (58). Three months after transplantation, the first SCD patient treated with plerixafor-mobilized transduced $CD34^+$ cells showed a significant level of HbF, accounting for 23% of total Hb and 60% of RBCs expressed HbF: these parameters are particularly important since the pancellular distribution of HbF and the amount of anti-sickling hemoglobin/per cell are two key criteria to obtain clinical benefit (59).

Future challenges: reducing the price and conditioning-related toxicity of gene therapy

Although the clinical trials have been opened worldwide, and the pre-clinical studies for addressing the accumulating issues identified in the previous clinical trials have shown successful outcomes, the high cost of current *ex vivo* HSC gene therapy is one of the major barrier to a widespread application of gene therapy to β-hemoglobinopathies (Fig. 1A). The main factors driving the high cost are that a large amount of GMP vector production is required to adequately transduce HSC. The use of transduction enhancers (60–63) might reduce the amount of vector required to achieve a good HSC transduction efficiency. In addition, novel and more effective LVs that correct the β-thalassemia and SCD phenotype with a low VCN per cell could also decrease the need of large volumes of viral preparations. Recently, one stable producer cell line has been described for a SCID-X1 lentiviral-based gene therapy trial (64) and if available, it should represent another step forward to reduce the cost of these viral preparations. Finally, genome editing-based strategies might be less expensive: manufacturing costs of clinical-grade edited cells are likely lower than those associated with LV-based approaches.

Partial myeloablative conditioning regimens could be another promising option to address high costs and reduce the conditioning-related toxicity (Fig. 1B). To secure the space in the bone marrow for the transduced β-thalassemia and SCD HSCs, complete myeloablative regimen might be required for success of gene therapy. Successful gene therapy trials have generally utilized myeloablative doses of busulfan around 12–16 mg/kg (65). These regimens appear to carry risks of both early transplant-associated toxicity as well as late effects like infertility and secondary malignancies (66). Severe myelosuppression and chemotherapy-associated toxicities require intensive support including multiple blood transfusions, infection prophylaxis and hospitalization in a protected environment, resulting in high treatment costs. One approach to circumvent this problem is to use monoclonal antibodies to target antigens expressed on HSCs. The approach has targeted CD45, limited in its expression to hematopoietic cells, or CD117 (also known as c-kit), expressed on HSCs, mast cells, melanocytes and other non-hematopoietic lineages. An antibody that recognizes mouse and human CD45, when coupled to the toxin saponin (CD45-SAP), enables long-term engraftment of syngeneic cells, while minimizing the cytopenia and immunosuppression due to toxic conditioning (67). Targeting CD117 enables autologous engraftment in immunodeficient mice (68), and when coupled with CD47 blockade enables engraftment in immunocompetent mice (69). Further clinical development of less toxic conditioning regimens will allow their use in a variety of nonmalignant indications for autologous or even allo-HSCT.

Another option to avoid the use of chemotherapy reagents for myeloablation is *in vivo* HSCs transduction, namely the direct injection of the vector into the body. *In vivo* HSC gene transfer achieved in a minimally invasive manner and without the need for stem cell harvest or transplantation would simplify the procedure and reduce the cost of this therapy. Wang *et al.* (70) reported that a single intraosseous infusion of LVs to hemophilia

mouse model was sufficient to correct phenotype for long term. Intravenous injection has also been shown as an alternative option to deliver vectors to HSCs in bone marrow. However, current VSV-G-pseudotyped LVs are not suitable for direct *in vivo* injection, since VSV_G is rapidly inactivated by human complements (71). In this respect, several pseudotyping envelopes (72) or different type of viral vectors [foamy virus (73,74) or adeno-associated viral vectors (75)] have emerged in the field. Moreover, *in vivo* injection triggers innate and adaptive immune responses. To solve these immune-related issues, more rigorous efficiency and safety studies are required. However, these novel exciting methods to deliver vectors are promising, and new reports are awaited.

As we reviewed here, clinical trials for hemoglobinopathies have shown promising outcomes so far. However, there are still issues that need to be solved, including the partial efficacy in severe β-thalassemia and SCD patients, toxicity (e.g. due to the conditioning regimen and potentially to insertional mutagenesis) and the high costs. Additional studies are needed to advance the technology to improve outcomes and address these issues.

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References

1. Ho, P.J. and Thein, S.L. (2000) Gene regulation and deregulation: a beta globin perspective. *Blood Rev.*, **14**, 78–93.
2. David Weatherall, B.G.F., Higgs, D.R. and Steinberg, M.H. (2009) *Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management*, 2nd edn. Cambridge Medicine (Hardcover) Cambridge University Press, in press.
3. Locatelli, F., Kabbara, N., Ruggeri, A., Ghavamzadeh, A., Roberts, I., Li, C.K., Bernaudin, F., Vermylen, C., Dalle, J.H., Stein, J. *et al.* (2013) Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling. *Blood*, **122**, 1072–1078.
4. Hulbert, M.L. and Shenoy, S. (2018) Hematopoietic stem cell transplantation for sickle cell disease: progress and challenges. *Pediatr. Blood Cancer*, **65**, e27263.
5. Bolanos-Meade, J., Fuchs, E.J., Luznik, L., Lanzkron, S.M., Gamper, C.J., Jones, R.J. and Brodsky, R.A. (2012) HLA-haploidentical bone marrow transplantation with post-transplant cyclophosphamide expands the donor pool for patients with sickle cell disease. *Blood*, **120**, 4285–4291.
6. de la Fuente, J., Dhedin, N., Koyama, T., Bernaudin, F., Kuentz, M., Karnik, L., Socie, G., Culos, K.A., Brodsky, R.A., DeBaun, M.R. *et al.* (2018) Haploidentical bone marrow transplantation with post-transplantation cyclophosphamide plus Thiotepa improves donor engraftment in patients with sickle cell anemia: results of an international learning collaborative. *Biol. Blood Marrow Transplant.*, **25**, 1197–1209.
7. Anurathapan, U., Hongeng, S., Pakakasama, S., Sirachainan, N., Songdej, D., Chuansumrit, A., Charoenkwan, P., Jetsrisuparb, A., Sanpakit, K., Rujkijyanont, P. *et al.* (2016) Hematopoietic stem cell transplantation for homozygous beta-thalassemia and beta-thalassemia/hemoglobin E patients from haploidentical donors. *Bone Marrow Transplant.*, **51**, 813–818.
8. Sodani, P., Isgro, A., Gaziev, J., Paciaroni, K., Marziali, M., Simone, M.D., Roveda, A., De Angelis, G., Gallucci, C., Torelli, F. *et al.* (2011) T cell-depleted hla-haploidentical stem cell transplantation in thalassemia young patients. *Pediatr. Rep.*, **3** (Suppl 2), e13.
9. Gaziev, J., Isgro, A., Sodani, P., Paciaroni, K., De Angelis, G., Marziali, M., Ribersani, M., Alfieri, C., Lanti, A., Galluccio, T. *et al.* (2018) Haploidentical HSCT for hemoglobinopathies: improved outcomes with TCRalphabeta(+)/CD19(+)-depleted grafts. *Blood Adv.*, **2**, 263–270.
10. Booth, C., Gaspar, H.B. and Thrasher, A.J. (2016) Treating immunodeficiency through HSC gene therapy. *Trends Mol. Med.*, **22**, 317–327.
11. Naldini, L. (2015) Gene therapy returns to centre stage. *Nature*, **526**, 351–360.
12. Cavazzana, M., Bushman, F.D., Miccio, A., Andre-Schmutz, I. and Six, E. (2019) Gene therapy targeting haematopoietic stem cells for inherited diseases: progress and challenges. *Nat. Rev. Drug Discov.*, **18**, 447–462.
13. Wilber, A., Nienhuis, A.W. and Persons, D.A. (2011) Transcriptional regulation of fetal to adult hemoglobin switching: new therapeutic opportunities. *Blood*, **117**, 3945–3953.
14. Smith, E.C. and Orkin, S.H. (2016) Hemoglobin genetics: recent contributions of GWAS and gene editing. *Hum. Mol. Genet.*, **25**, R99–R105.
15. Dong, A.C. and Rivella, S. (2017) Gene addition strategies for beta-thalassemia and sickle cell anemia. *Adv. Exp. Med. Biol.*, **1013**, 155–176.
16. Tuan, D., Solomon, W., Li, Q. and London, I.M. (1985) The 'beta-like-globin' gene domain in human erythroid cells. *Proc. Natl. Acad. Sci. U. S. A.*, **82**, 6384–6388.
17. Cavazzana-Calvo, M., Payen, E., Negre, O., Wang, G., Hehir, K., Fusil, F., Down, J., Denaro, M., Brady, T., Westerman, K. *et al.* (2010) Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. *Nature*, **467**, 318–322.
18. Ribeil, J.A., Hacein-Bey-Abina, S., Payen, E., Magnani, A., Semeraro, M., Magrin, E., Caccavelli, L., Neven, B., Bourget, P., El Nemer, W. *et al.* (2017) Gene therapy in a patient with sickle cell disease. *N. Engl. J. Med.*, **376**, 848–855.
19. Thompson, A.A., Walters, M.C., Kwiatkowski, J., Rasko, J.E.J., Ribeil, J.A., Hongeng, S., Magrin, E., Schiller, G.J., Payen, E., Semeraro, M. *et al.* (2018) Gene therapy in patients with transfusion-dependent beta-thalassemia. *N. Engl. J. Med.*, **378**, 1479–1493.
20. Morrison, C. (2015) $1-million price tag set for Glybera gene therapy. *Nat. Biotechnol.*, **33**, 217–218.
21. Darrow, J.J. (2019) Luxturna: FDA documents reveal the value of a costly gene therapy. *Drug Discov. Today*, **24**, 949–954.
22. Patel, U., Boucher, M., de Leseleuc, L. and Visintini, S. (2016) *CADTH Issues in Emerging Health Technologies*, Ottawa (ON), in press, pp. 1–11.
23. Wada-Kiyama, Y. and Kiyama, R. (1994) Periodicity of DNA bend sites in human epsilon-globin gene region. Possibility

of sequence-directed nucleosome phasing. *J. Biol. Chem.*, **269**, 22238–22244.
24. Novak, U., Harris, E.A., Forrester, W., Groudine, M. and Gelinas, R. (1990) High-level beta-globin expression after retroviral transfer of locus activation region-containing human beta-globin gene derivatives into murine erythroleukemia cells. *Proc. Natl. Acad. Sci. U. S. A.*, **87**, 3386–3390.
25. Karlsson, S., Bodine, D.M., Perry, L., Papayannopoulou, T. and Nienhuis, A.W. (1988) Expression of the human beta-globin gene following retroviral-mediated transfer into multipotential hematopoietic progenitors of mice. *Proc. Natl. Acad. Sci. U. S. A.*, **85**, 6062–6066.
26. Dzierzak, E.A., Papayannopoulou, T. and Mulligan, R.C. (1988) Lineage-specific expression of a human beta-globin gene in murine bone marrow transplant recipients reconstituted with retrovirus-transduced stem cells. *Nature*, **331**, 35–41.
27. Cone, R.D., Weber-Benarous, A., Baorto, D. and Mulligan, R.C. (1987) Regulated expression of a complete human beta-globin gene encoded by a transmissible retrovirus vector. *Mol. Cell. Biol.*, **7**, 887–897.
28. May, C., Rivella, S., Callegari, J., Heller, G., Gaensler, K.M., Luzzatto, L. and Sadelain, M. (2000) Therapeutic haemoglobin synthesis in beta-thalassaemic mice expressing lentivirus-encoded human beta-globin. *Nature*, **406**, 82–86.
29. Pawliuk, R., Westerman, K.A., Fabry, M.E., Payen, E., Tighe, R., Bouhassira, E.E., Acharya, S.A., Ellis, J., London, I.M., Eaves, C.J. *et al.* (2001) Correction of sickle cell disease in transgenic mouse models by gene therapy. *Science*, **294**, 2368–2371.
30. May, C., Rivella, S., Chadburn, A. and Sadelain, M. (2002) Successful treatment of murine beta-thalassemia intermedia by transfer of the human beta-globin gene. *Blood*, **99**, 1902–1908.
31. Rivella, S., May, C., Chadburn, A., Riviere, I. and Sadelain, M. (2003) A novel murine model of Cooley anemia and its rescue by lentiviral-mediated human beta-globin gene transfer. *Blood*, **101**, 2932–2939.
32. Perrine, R.P., Brown, M.J., Clegg, J.B., Weatherall, D.J. and May, A. (1972) Benign sickle-cell anaemia. *Lancet*, **2**, 1163–1167.
33. Platt, O.S., Brambilla, D.J., Rosse, W.F., Milner, P.F., Castro, O., Steinberg, M.H. and Klug, P.P. (1994) Mortality in sickle cell disease. Life expectancy and risk factors for early death. *N. Engl. J. Med.*, **330**, 1639–1644.
34. Bunn, H.F. (1997) Pathogenesis and treatment of sickle cell disease. *N. Engl. J. Med.*, **337**, 762–769.
35. Charache, S., Terrin, M.L., Moore, R.D., Dover, G.J., Barton, F.B., Eckert, S.V., McMahon, R.P. and Bonds, D.R. (1995) Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the multicenter study of hydroxyurea in sickle cell anemia. *N. Engl. J. Med.*, **332**, 1317–1322.
36. Uda, M., Galanello, R., Sanna, S., Lettre, G., Sankaran, V.G., Chen, W., Usala, G., Busonero, F., Maschio, A., Albai, G. *et al.* (2008) 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.*, **105**, 1620–1625.
37. Menzel, S., Garner, C., Gut, I., Matsuda, F., Yamaguchi, M., Heath, S., Foglio, M., Zelenika, D., Boland, A., Rooks, H. *et al.* (2007) A QTL influencing F cell production maps to a gene encoding a zinc-finger protein on chromosome 2p15. *Nat. Genet.*, **39**, 1197–1199.
38. Bauer, D.E., Kamran, S.C., Lessard, S., Xu, J., Fujiwara, Y., Lin, C., Shao, Z., Canver, M.C., Smith, E.C., Pinello, L. *et al.* (2013) An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. *Science*, **342**, 253–257.
39. Funnell, A.P., Prontera, P., Ottaviani, V., Piccione, M., Giambona, A., Maggio, A., Ciaffoni, F., Stehling-Sun, S., Marra, M., Masiello, F. *et al.* (2015) 2p15-p16.1 microdeletions encompassing and proximal to BCL11A are associated with elevated HbF in addition to neurologic impairment. *Blood*, **126**, 89–93.
40. Liu, P., Keller, J.R., Ortiz, M., Tessarollo, L., Rachel, R.A., Nakamura, T., Jenkins, N.A. and Copeland, N.G. (2003) Bcl11a is essential for normal lymphoid development. *Nat. Immunol.*, **4**, 525–532.
41. Ippolito, G.C., Dekker, J.D., Wang, Y.H., Lee, B.K., Shaffer, A.L., 3rd, Lin, J., Wall, J.K., Lee, B.S., Staudt, L.M., Liu, Y.J. *et al.* (2014) Dendritic cell fate is determined by BCL11A. *Proc. Natl. Acad. Sci. U. S. A.*, **111**, E998–E1006.
42. Luc, S., Huang, J., McEldoon, J.L., Somuncular, E., Li, D., Rhodes, C., Mamoor, S., Hou, S., Xu, J. and Orkin, S.H. (2016) Bcl11a deficiency leads to hematopoietic stem cell defects with an aging-like phenotype. *Cell Rep.*, **16**, 3181–3194.
43. Tsang, J.C., Yu, Y., Burke, S., Buettner, F., Wang, C., Kolodziejczyk, A.A., Teichmann, S.A., Lu, L. and Liu, P. (2015) Single-cell transcriptomic reconstruction reveals cell cycle and multi-lineage differentiation defects in Bcl11a-deficient hematopoietic stem cells. *Genome Biol.*, **16**, 178.
44. Sankaran, V.G., Menne, T.F., Xu, J., Akie, T.E., Lettre, G., Van Handel, B., Mikkola, H.K., Hirschhorn, J.N., Cantor, A.B. and Orkin, S.H. (2008) Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. *Science*, **322**, 1839–1842.
45. Guda, S., Brendel, C., Renella, R., Du, P., Bauer, D.E., Canver, M.C., Grenier, J.K., Grimson, A.W., Kamran, S.C., Thornton, J. *et al.* (2015) miRNA-embedded shRNAs for lineage-specific BCL11A knockdown and hemoglobin F induction. *Mol. Ther.*, **23**, 1465–1474.
46. Canver, M.C., Smith, E.C., Sher, F., Pinello, L., Sanjana, N.E., Shalem, O., Chen, D.D., Schupp, P.G., Vinjamur, D.S., Garcia, S.P. *et al.* (2015) BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis. *Nature*, **527**, 192–197.
47. Chang, K.H., Smith, S.E., Sullivan, T., Chen, K., Zhou, Q., West, J.A., Liu, M., Liu, Y., Vieira, B.F., Sun, C. *et al.* (2017) 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.*, **4**, 137–148.
48. Psatha, N., Reik, A., Phelps, S., Zhou, Y., Dalas, D., Yannaki, E., Levasseur, D.N., Urnov, F.D., Holmes, M.C. and Papayannopoulou, T. (2018) Disruption of the BCL11A erythroid enhancer reactivates fetal hemoglobin in erythroid cells of patients with beta-thalassemia major. *Mol. Ther. Methods Clin. Dev.*, **10**, 313–326.
49. Wu, Y., Zeng, J., Roscoe, B.P., Liu, P., Yao, Q., Lazzarotto, C.R., Clement, K., Cole, M.A., Luk, K., Baricordi, C. *et al.* (2019) Highly efficient therapeutic gene editing of human hematopoietic stem cells. *Nat. Med.*, **25**, 776–783.
50. Cattoglio, C., Facchini, G., Sartori, D., Antonelli, A., Miccio, A., Cassani, B., Schmidt, M., von Kalle, C., Howe, S., Thrasher, A.J. *et al.* (2007) Hot spots of retroviral integration in human CD34+ hematopoietic cells. *Blood*, **110**, 1770–1778.
51. Marktel, S., Scaramuzza, S., Cicalese, M.P., Giglio, F., Galimberti, S., Lidonnici, M.R., Calbi, V., Assanelli, A., Bernardo, M.E., Rossi, C. *et al.* (2019) Intrabone hematopoietic

stem cell gene therapy for adult and pediatric patients affected by transfusion-dependent ss-thalassemia. *Nat. Med.*, **25**, 234–241.
52. Rasko, J.E.J., Thompson, A.T., Kwiatkowski, J.L., Hongeng, S., Schiller, G.J., Anurathapan, U., Cavazzana, M., Ho, P.J., Schmidt, M., Morris Kletzel, M. *et al.* (2018) *American Society of Hematology. American Society of Hematology*, San Diego Vol. Blood 132 no. Suppl 1 167, pp. 132–167.
53. Breda, L., Casu, C., Gardenghi, S., Bianchi, N., Cartegni, L., Narla, M., Yazdanbakhsh, K., Musso, M., Manwani, D., Little, J. *et al.* (2012) Therapeutic hemoglobin levels after gene transfer in beta-thalassemia mice and in hematopoietic cells of beta-thalassemia and sickle cells disease patients. *PLoS One*, **7**, e32345.
54. Tisdale, J., Kanter, J., Mapara, M.Y., Kwiatkowski, J., Krishnamurti, L., Schmidt, M., Miller, A.L., Pierciey Jr, F.J., Shi, W., Ribeil, J.A. *et al.* (2018) Current results of lentiglobin gene therapy in patients with severe sickle cell disease treated under a refined protocol in the phase 1 Hgb-206 study. in press.
55. Kanter, J., T.J., Kwiatkowski, J. *et al.* (2018) Outcomes for initial patient cohorts with up to 33 months of follow-up in the Hgb-206 phase 1 trial. in press.
56. Lagresle-Peyrou, C., Lefrere, F., Magrin, E., Ribeil, J.A., Romano, O., Weber, L., Magnani, A., Sadek, H., Plantier, C., Gabrion, A. *et al.* (2018) Plerixafor enables safe, rapid, efficient mobilization of hematopoietic stem cells in sickle cell disease patients after exchange transfusion. *Haematologica*, **103**, 778–786.
57. Malik, P., Grimley, M., Quinn, C.T., Shova, A., Little, C., Lutzko, C., Kalfa, T.A., Niss, O., Mehta, P.A., Chandra, S. *et al.* (2018) Gene therapy for sickle cell anemia using a modified gamma globin Lentivirus vector and reduced intensity conditioning transplant shows promising correction of the disease phenotype. *Blood*, **132**, 1021.
58. Brendel, C., Guda, S., Renella, R., Bauer, D.E., Canver, M.C., Kim, Y.J., Heeney, M.M., Klatt, D., Fogel, J., Milsom, M.D. *et al.* (2016) Lineage-specific BCL11A knockdown circumvents toxicities and reverses sickle phenotype. *J. Clin. Invest.*, **126**, 3868–3878.
59. Esrick, E.B., Brendel, C., Manis, J.P., Armant, M.A., Negre, H., Dansereau, C., Ciuculescu, M.F., Patriarca, S., Mackinnon, B., Daley, H. *et al.* (2018) Flipping the switch: initial results of genetic targeting of the fetal to adult globin switch in sickle cell patients. *Blood*, in press.
60. Petrillo, C., Cesana, D., Piras, F., Bartolaccini, S., Naldini, L., Montini, E. and Kajaste-Rudnitski, A. (2015) Cyclosporin a and rapamycin relieve distinct lentiviral restriction blocks in hematopoietic stem and progenitor cells. *Mol. Ther.*, **23**, 352–362.
61. Zonari, E., Desantis, G., Petrillo, C., Boccalatte, F.E., Lidonnici, M.R., Kajaste-Rudnitski, A., Aiuti, A., Ferrari, G., Naldini, L. and Gentner, B. (2017) Efficient ex vivo engineering and expansion of highly purified human hematopoietic stem and progenitor cell populations for gene therapy. *Stem Cell Reports*, **8**, 977–990.
62. Heffner, G.C., Bonner, M., Christiansen, L., Pierciey, F.J., Campbell, D., Smurnyy, Y., Zhang, W., Hamel, A., Shaw, S., Lewis, G. *et al.* (2018) Prostaglandin E2 increases lentiviral vector transduction efficiency of adult human hematopoietic stem and progenitor cells. *Mol. Ther.*, **26**, 320–328.
63. Wang, C.X., Sather, B.D., Wang, X., Adair, J., Khan, I., Singh, S., Lang, S., Adams, A., Curinga, G., Kiem, H.P. *et al.* (2014) Rapamycin relieves lentiviral vector transduction resistance in human and mouse hematopoietic stem cells. *Blood*, **124**, 913–923.
64. Mamcarz, E., Zhou, S., Lockey, T., Abdelsamed, H., Cross, S.J., Kang, G., Ma, Z., Condori, J., Dowdy, J., Triplett, B. *et al.* (2019) Lentiviral gene therapy combined with low-dose Busulfan in infants with SCID-X1. *NEJM*, **18**, 1525–1534.
65. Cowan, M.J., Dvorak, C.C. and Long-Boyle, J. (2017) Opening marrow niches in patients undergoing autologous hematopoietic stem cell gene therapy. *Hematol. Oncol. Clin. North Am.*, **31**, 809–822.
66. Allewelt, H., El-Khorazaty, J., Mendizabal, A., Taskindoust, M., Martin, P.L., Prasad, V., Page, K., Sanders, J. and Kurtzberg, J. (2016) Late effects after umbilical cord blood transplantation in very young children after Busulfan-based, myeloablative conditioning. *Biol. Blood Marrow Transplant.*, **22**, 1627–1635.
67. Palchaudhuri, R., Saez, B., Hoggatt, J., Schajnovitz, A., Sykes, D.B., Tate, T.A., Czechowicz, A., Kfoury, Y., Ruchika, F., Rossi, D.J. *et al.* (2016) Non-genotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin. *Nat. Biotechnol.*, **34**, 738–745.
68. Czechowicz, A., Kraft, D., Weissman, I.L. and Bhattacharya, D. (2007) Efficient transplantation via antibody-based clearance of hematopoietic stem cell niches. *Science*, **318**, 1296–1299.
69. Chhabra, A., Ring, A.M., Weiskopf, K., Schnorr, P.J., Gordon, S., Le, A.C., Kwon, H.S., Ring, N.G., Volkmer, J., Ho, P.Y. *et al.* (2016) Hematopoietic stem cell transplantation in immunocompetent hosts without radiation or chemotherapy. *Sci. Transl. Med.*, **8**, 351ra105.
70. Wang, X., Shin, S.C., Chiang, A.F., Khan, I., Pan, D., Rawlings, D.J. and Miao, C.H. (2015) Intraosseous delivery of lentiviral vectors targeting factor VIII expression in platelets corrects murine hemophilia A. *Mol. Ther.*, **23**, 617–626.
71. Frecha, C., Fusil, F., Cosset, F.L. and Verhoeyen, E. (2011) In vivo gene delivery into hCD34+ cells in a humanized mouse model. *Methods Mol. Biol.*, **737**, 367–390.
72. Joglekar, A.V. and Sandoval, S. (2017) Pseudotyped lentiviral vectors: one vector, many guises. *Hum. Gene Ther. Methods*, **28**, 291–301.
73. Russell, D.W. and Miller, A.D. (1996) Foamy virus vectors. *J. Virol.*, **70**, 217–222.
74. Burtner, C.R., Beard, B.C., Kennedy, D.R., Wohlfahrt, M.E., Adair, J.E., Trobridge, G.D., Scharenberg, A.M., Torgerson, T.R., Rawlings, D.J., Felsburg, P.J. *et al.* (2014) Intravenous injection of a foamy virus vector to correct canine SCID-X1. *Blood*, **123**, 3578–3584.
75. Kay, M.A., Manno, C.S., Ragni, M.V., Larson, P.J., Couto, L.B., McClelland, A., Glader, B., Chew, A.J., Tai, S.J., Herzog, R.W. *et al.* (2000) Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector. *Nat. Genet.*, **24**, 257–261.