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Hemoglobin disorders: lentiviral gene therapy in the starting blocks to enter clinical practice

Karine Sii-Felice[a], Marie Giorgi[a], Philippe Leboulch[a,b,c], and Emmanuel Payen[a,d]

[a]*UMR E007, Service of Innovative Therapies, Institute of Biology François Jacob and University Paris Saclay, CEA Paris Saclay, Fontenay-aux-Roses, France;* [b]*Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA;* [c]*Ramathibodi Hospital, Mahidol University, Bangkok, Thailand;* [d]*INSERM, Paris, France*

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The β-hemoglobinopathies, transfusion-dependent β-thalassemia and sickle cell disease, are the most prevalent inherited disorders worldwide and affect millions of people. Many of these patients have a shortened life expectancy and suffer from severe morbidity despite supportive therapies, which impose an enormous financial burden to societies. The only available curative therapy is allogeneic hematopoietic stem cell transplantation, although most patients do not have an HLA-matched sibling donor, and those who do still risk life-threatening complications. Therefore, gene therapy by one-time ex vivo modification of hematopoietic stem cells followed by autologous engraftment is an attractive new therapeutic modality. The first proof-of-principle of conversion to transfusion independence by means of a lentiviral vector expressing a marked and anti-sickling β^{T87Q}-globin gene variant was reported a decade ago in a patient with transfusion-dependent β-thalassemia. In follow-up multicenter Phase II trials with an essentially identical vector (termed LentiGlobin BB305) and protocol, 12 of the 13 patients with a non-β^0/β^0 genotype, representing more than half of all transfusion-dependent β-thalassemia cases worldwide, stopped red blood cell transfusions with total hemoglobin levels in blood approaching normal values. Correction of biological markers of dyserythropoiesis was achieved in evaluated patients. In nine patients with β^0/β^0 transfusion-dependent β-thalassemia or equivalent severity ($\beta^{IVS1-110}$), median annualized transfusion volume decreased by 73% and red blood cell transfusions were stopped in three patients. Proof-of-principle of therapeutic efficacy in the first patient with sickle cell disease was also reported with LentiGlobin BB305. Encouraging results were presented in children with transfusion-dependent β-thalassemia in another trial with the GLOBE lentiviral vector and several other gene therapy trials are currently open for both transfusion-dependent β-thalassemia and sickle cell disease. Phase III trials are now under way and should help to determine benefit/risk/cost ratios to move gene therapy toward clinical practice.

The β-hemoglobinopathies, β-thalassemia (β-thal) and sickle cell disease (SCD), are the most common monogenic diseases worldwide. They result from mutations in the β-globin (*HBB*) gene locus that lead to the production of insufficient (β^0, β^+) or aberrant (β^S) β-globin protein. In β-thal, profound anemia results from absent or insufficient hemoglobin (Hb) concentration within red blood cells (RBCs) together with the toxic effects of unpaired free β-globin for RBC membranes (hemichrome precipitation), which triggers ineffective erythropoiesis and chronic hemolysis. The most severe clinical form of β-thal is transfusion-dependent β-thal (TDT), in which patients depend on chronic RBC transfusions for the prevention of lethal complications and survival. More than 200 mutations have been found to result in β-thal and the genotypes responsible for approximately one-half of all TDT worldwide are β^E/β^0, the other half being caused by other β^+ mutations or β^0/β^0-thal.

The molecular basis for SCD is the S mutation, a single base substitution (A to T) in the sixth exon of

Offprint requests to: Emmanuel Payen, Institut de Biologie François Jacob, CEA Paris Saclay/Site de Fontenay aux Roses, 18 route du Panorama BP6, 92265 Fontenay aux Roses Cedex, France; E-mail: emmanuel.payen@cea.fr

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the *HBB* gene that results in Glu6Val amino acid substitution. Sickle hemoglobin (HbS) polymerizes upon deoxygenation, reducing RBC deformability and modifying their adhesion properties, ultimately resulting in intensely painful vaso-occlusive crises (VOCs) and acute chest syndrome (ACS), irreversible organ damage, poor quality of life, and reduced life expectancy. Severe SCD genotypes are represented by homozygous β^S/β^S and heterozygous β^S/β^0.

Inherited Hb disorders: an increasing global health burden

At least 300,000 babies are born with a severe inherited Hb disorder each year worldwide [1], resulting in a global patient population in the tens of millions and accounting for ~3.4% of deaths in children under the age of 5 years, mostly in Africa [1,2]. The prevalence of β-thal is 1–20% in North Africa, the Mediterranean basin, the Middle East, the Indian subcontinent, southern China, southeast Asia, Melanesia, and the southwest Pacific islands [3]. The β^S mutation is widespread throughout sub-Saharan Africa, the Middle East, and parts of the Indian subcontinent, with carrier frequencies of 5–40% [3]. These disorders were originally restricted to tropical and subtropical regions, where carriers are protected against dying from falciparum malaria [4,5]. However, because of increasing mobility and migration from endemic areas [6–8], the β-hemoglobinopathies are increasingly common in the non-endemic countries of northern and western Europe [8, 9], North America [10], South America [11], and Australia [12]. The prevalence of Hb disorders ranges from 0.3 to 25 per 1000 live births worldwide [13]. These two severe diseases are associated with a shortened life expectancy and a number of comorbid conditions affecting emotional, physical, mental, and societal aspects of a patient's life [14–19].

The high frequency of Hb disorders and the large number of patients are likely to persist or even increase further [20]. The resurgence of malaria in many parts of the world [21,22] is likely to maintain a high carrier frequency. Efficient malaria control programs [23] will need to be maintained in the long term for any significant decrease to be anticipated [24]. Disease prevention through premarital screening and genetic counseling [25–28] and/or antenatal detection programs [29–31] has been implemented successfully in a few countries [32], but it has had little global impact [13] due to economic or cultural constraints in many endemic regions [30,33] and ongoing debate about the value of population-wide screening [34]. The demographic transition is continuing and childhood mortality rates are falling due to improvements in hygiene, nutrition, and infection control [35]. Many patients with Hb disorders, who would previously have died in infancy, are now surviving into adulthood in low- to middle-income countries. The β-hemoglobinopathies thus constitute a growing burden on health services in many nations [24]. According to the World Health Organization, Hb disorders constitute an increasing health problem in 71% of countries [36].

In countries where modern medical techniques are available, the life expectancy of patients with TDT has extended into adulthood [37,38]. Most of these patients are treated with lifelong supportive therapy. Disease management, which includes regular follow-up visits, blood transfusion, iron chelation, laboratory tests, and treatment of side effects, is costly and represents a significant financial burden to health services. The cost of lifetime treatment has been estimated at near £220,000/patient in the United Kingdom in 1999 [39], US$280,000/patient in Israel [40] in 1998, and US$150,000/patient in Thailand in 2002 [41]. More recent studies have estimated costs at €15,000/patient/year in Italy in 2006 [42], US$40,000/patient/year in Israel [31] in 2013, and near US$50,000/patient/year in Australia in 2015 [43]. Factors accounting for these differences include an increase in costs after the introduction of the new oral iron chelator deferasirox, efforts to increase quality of life, and the progressive increase in the life expectancy of patients with TDT. In Cyprus, treatment costs would currently exceed the national health budget if strict measures to control the number of affected births had not been taken in the 1970s [3]. In low-/middle-income countries, optimal care clearly outstrips health resources.

Newborn screening, the early implementation of comprehensive care, and parental education [44] have greatly increased the survival of young children and adolescents with SCD in high-income countries [45–49]. This has shifted the burden of mortality into early adulthood [50]. SCD is now the most common inherited genetic disorder in France, with an overall prevalence of 1/2221 newborns in 2013 [51], which is ~15,000 patients and 1 in 800 live births affected in the Parisian region. Frequencies are similar in the United Kingdom [52] and slightly higher in the United States, where ~1 in 1900 babies [53] are affected by SCD overall and 1 in 400 babies in the African-American population [54]. Most of the individuals treated with hydroxyurea now live into their forties [55]. Data from a Belgian cohort indicate that 99% of children with SCD treated with hydroxyurea therapy had the potential to reach their 15th birthday [56]. Nevertheless, these patients have many complications, some of which are life-threatening or greatly impair health-related quality of life [57]. Despite improvements in the treatment and management of SCD, 10% of affected children remain at high risk of mortality and are eligible for hematopoietic stem cell transplantation

(HSCT). Brain hemorrhage remains the leading cause of death, followed by infection. The average lifetime cost of care for SCD in the United States was estimated at US$460,000/patient in 2005 [58].

Rationale for gene therapy by gene addition

Notwithstanding improvements in life expectancy on conventional therapies, HSCT remains the only curative treatment for patients with TDT [59] and severe SCD [60]. According to an international panel of experts, every young patient with TDT and SCD for whom an HLA-matched sibling donor (MSD) is available should be offered HSCT before iron overload and/or severe complications occur [61], although transplantation-associated mortality and graft failure frequencies are not negligible, at 2–10% and 5–15%, respectively [62,63]. Patients with TDT [64–66] and SCD [67] have a better health status after transplantation than those on conventional therapies. These conclusions, based on medical criteria, render any comparisons of costs with noncurative therapy cynical. Nevertheless, in a context in which the proportion of nations' budgets dedicated to health is increasing more rapidly than that of any other component, it is important to evaluate the cost per life-year gained relative to palliative therapies in an effort to convince governments and payers of the advantages of HSCT for societies as a whole [68,69]. Based on the current life expectancy of patients with TDT and SCD in industrialized countries, HSCT from sibling donors in children, the median cost of which is between US$100,000 and US$150,000 [68], is cost-effective relative to lifelong supportive therapy [70].

Other than in countries in which family size is generally large [71], less than 25–30% of patients have an available MSD [72]. HSCT from donors that are either adult matched unrelated donors (MUD), partially HLA-mismatched, or related HLA-haploidentical can be used as an alternative to MSD. The introduction of high-resolution molecular HLA-typing techniques has made it possible to achieve outcomes for MUD transplantation similar to those achieved with transplantation of cells from a sibling in a limited series of TDT patients [59,73], although this is not yet possible for patients with SCD [74]. The risk of graft-versus-host disease (GVHD) is much higher than that in the MSD setting and is considered unacceptable [74,75]. Patients with chronic GVHD have a much lower level of post-transplantation quality of life than other patients [65,66,76] and, despite prophylaxis, GVHD is the main treatment-related cause of death in cases of unrelated HSCT [75,77]. Immunoablative reduced-intensity conditioning is an interesting strategy to reduce transplantation-related mortality and morbidity, especially in high-risk or elderly thalassemia patients. However, this strategy is still under investigation [78,79]. Nobel laureate Dr. Edward Donnall Thomas, a pioneer in stem cell research and bone marrow transplantation [80,81], was one of the first to argue that HSCT represents a form of natural gene therapy because all of the donor genes are put into the recipient. However, for those with no MSD and those at high risk of transplantation-related morbidity, gene therapy with autologous corrected HSCs is a highly promising alternative.

Precision gene editing by homology-directed repair (HDR) within the HBB locus using engineered nucleases and donor templates is theoretically an ideal method for repairing the patient's HSC safely and ensuring optimal β-globin expression. However, low rates of HDR in HSCs [82], the inefficient delivery of nucleases and templates [83], potential off-target/oncotarget cleavage [84], and the low engraftment potential of HSCs with repaired genes [85] are all issues that need to be addressed further before this approach can be translated into clinical practice [86]. In addition, the very large number of mutations (>200 for β-thal) observed in patients would require an equally large number of HDR products receiving regulatory and market approval unless one focuses only on the most common genotypes.

For all of these reasons, ex vivo HSC modification by gene addition remains the approach of choice for the gene therapy of the β-hemoglobinopathies [87]. Decades of research leading to the identification, isolation, reduction, and blending of β-globin regulatory elements, especially the β-locus control region (LCR) and its DNase I hypersensitive sites [88,89], and the advent of lentiviral vectors (LVs) capable of conveying large genomic sequences into nondividing cells, including HSCs [90], have made this approach possible. We and others have demonstrated the therapeutic capacity of β-globin-expressing LVs to correct mouse models of β-thal and SCD [91–94] and we have shown the safety of this approach in suitable models [95,96].

Clinical experience with gene addition for the β-hemoglobinopathies

LV expressing mutated β^{AT87Q}-globin

We have designed a family of LVs, referred to as LentiGlobin, which contain a mutated β^{AT87Q}-globin gene as the therapeutic payload. This gene presents distinct advantages for the gene therapy of both SCD and TDT, as reviewed below and in the section "For SCD."

Because wild-type human β-globin is relatively poorly inhibitor of HbS polymerization since it acts by mere dilution [97], we turned to β-globin variants to design the therapeutic product to be expressed by gene addition. Rather than using wild-type γ-globin, which interferes strongly with HbS polymerization [98], but

has oxygen-carrying and tetramer assembly properties that differ from β-globin [99], and because transferring and expressing the fetal γ-globin gene at high levels in "adult" RBCs poses specific challenges, we decided to replace a β-globin codon within the human β-globin gene with that responsible for most of the strong anti-sickling properties of γ-globin: β^{AT87Q} [100] (see section "For SCD") [91]. Furthermore, the use of such a mutated β^{AT87Q}-globin gene for the gene therapy of TDT makes it uniquely possible to quantify therapeutic gene expression differentially from endogenous or exogenous wild-type β-globin protein in those TDT patients who are either β^{+} or continue to be transfused [101].

We made use of the optimized human β-globin gene cassette we had reported previously for stable transfer in Moloney murine retroviral vectors [102], with specific 3′ and promoter boundaries, a 372 bp segmental deletion of intron 2, and segments of HS2, HS3, and HS4 of the LCR. Because of the increased gene transfer capacity of lentiviral vectors, the LCR segments were further extended [91,92]. After removal of the U3 segment from the right long terminal repeat (LTR), tandem copies of the core cHS4 chromatin insulator from the chicken β-globin locus were inserted where U3 was located, in an effort to restrict adjacent (onco) gene activation at the sites of chromosomal integration in transduced cells [95]. The LV so described was referred to as LentiGlobin HPV569 [103].

Gene therapy of TDT

First Phase I/II trial: proof-of-principle in a human patient. After setting up large-scale clinical grade (good manufacturing practices) manufacturing of the LentiGlobin HPV569 vector, establishing controlled testing and release criteria, performing a large body of preclinical studies in mice and human cells aiming at demonstrating safety and efficacy, and drafting a clinical protocol with standard operating procedures and strict inclusion and exclusion criteria and endpoints, we initiated the first clinical trial for TDT and SCD [103]. This trial (Study LG001), together with another for adrenoleukodystrophy [104], were the first human trials approved worldwide for the gene therapy of inherited disorders by means of LVs, so granted by the French regulatory authorities in 2006 (Table 1) [90].

The first treated subject enrolled in Study LG001 who did not receive back-up cells was a β^{E}/β^{0} TDT subject (Patient 1003) who had been transfusion dependent since early childhood and received 3.9×10^{6} transduced bone marrow $CD34^{+}$ cells/kg. The conditioning regimen involved the injection of busulfan at a myeloablative dose of 12.8 mg/kg. The vector copy number (VCN) in the product administered was 0.6 [103]. Transplantation was uneventful and the patient became transfusion independent 1 year after cell infusion [103,105,106]. Blood Hb levels were stable, at ~8–8.5g/dL, for more than 8 years, with HbA^{T87Q} accounting for ~30% of total Hb (HbE, HbF, and HbA^{T87Q}) and a mean VCN in peripheral blood mononucleated cells (PBMCs) of ~0.2 [87,103,107]. Recently, the patient underwent two transfusions with RBCs after experiencing mild clinical symptoms of anemia 8 years after transplantation [87], but VCN in PBMCs and HbA^{T87Q} levels remain largely unchanged to date, a decade after gene therapy [108].

In this patient, a dominant clone in which the LV was inserted into the HMGA2 gene was detected a few months after transplantation [103]. Its levels reached a plateau between years 1 and 3 at 25–50% of vector-bearing PBMCs (i.e., 5–10% of all PBMCs), declining thereafter [105]. As of 3 years ago, this clone represents < 2% of all PBMCs, with no decrease in total Hb levels [87,107,108]. By year 8 after gene therapy, HMGA2 IS only ranks 5^{th} in clonal contribution in this patient [108]. LV insertion into the HMGA2 gene caused transcription dysregulation of the gene, resulting in large amounts of active protein in the patient's erythroid cell [103]. A report that HMGA2 overexpression confers a selective advantage to mouse hematopoietic cells [109] suggests a probable causal link between LV insertion and clonal dominance in this patient, raising concerns about LV genotoxicity. Nevertheless, more than 8 years after gene therapy, there are no signs of clonal outgrowth or toxicity [87] and insertion sites near or within the HMGA2 gene were detected in other clinical studies without evidence of myeloproliferation [87]. Importantly, only very few transduced HSCs were infused in this patient, as evidenced by a number of unique integration sites (UIS) <30 at year 2 and 127 at year 8, likely due to the relative toxicity of non-chromatography-purified vector preparations. This phenomenon also accounted for the relative dominance of a few clones such as HMGA2. As discussed below with subsequent Phase II trials, this situation has completely changed with the advent of chromatography purification of LentiGlobin vectors, with which the number of UIS retrieved from subsequent patients is 10- to 100-fold greater than what was observed with LG001 Patient 1003.

Another β^{E}/β^{0}-TDT subject (Patient 1004) underwent successful transplantation with HPV569-transduced autologous stem cells in 2011 (Table 1), but VCN was lower than in Patient 1003 and no clinical benefit was achieved [87].

This early clinical trial of LV-transduced hematopoietic cells as a drug product provided invaluable proof-of-principle for the potential of LVs to change the life

Table 1. Human clinical trials to date for gene therapy of TDT and/or severe SCD with our LVs (HPV569 and then BB305)

Trial identifier Phase Location Start date	Condition Patients	Vector Gene	$CD34^+$ Collection	Cell Dose ($\times 10^6$/kg)	DP VCN	Conditioning	PB VCN	Sponsor	Comments	References
LG001 I/II France Sept. 2006	TDT : 2[a] SCD : 0[a]	HPV569 β^{A-T87Q}	BM or G	1003 : 3.9 1004 : 4.3	1003 : 0.6 1004 : 0.3	Busulfan (myeloablative)	1003 : 0.2 1004 : 0.016	bluebird bio	1 subject (1003) transfusion independent 8 years post infusion	[5,87,103, 105–108]
NCT02151526 (HGB–205) I/II France July 2013	TDT : 4 SCD : 3	BB305 β^{A-T87Q}	TDT: G/P SCD: BM	TDT: 8.8–13.6 SCD: 3–5.6	TDT: 0.8–2.1 SCD: 0.5–1.2	Busulfan (myeloablative)	TDT: 0.3–4.2 SCD: 0.3–2.3[b]	bluebird bio	– 4 TDT free of transfusion (the longest 3.8 years by December 2017). – Clinical benefit for the first patient with SCD (50% HbA^{T87Q}, 30 months post–GT). – 15% and 20% HbA^{T87Q} in the two recently included SCD patients	[108,110, 117–119,149]
NCT01745120 (HGB–204) I/II US, Aust, Thai Aug 2013	TDT: 18 β^0/β^0: 8 Non–β^0/β^0: 10	BB305 β^{A-T87Q}	G/P	5.2–18.1	0.3–1.5	Busulfan (myeloablative)	0.1–1.0	bluebird bio	9 patients with non–β^0/β^0 genotypes and 2 patients with β^0/β^0 genotypes free of transfusion	[108,111, 151,246]
NCT02140554 (HGB–206) I US Aug 2014	SCD: 29 recruiting	BB305 β^{A-T87Q}	Gr A: BM Gr B1: BM[f] Gr B2: BM/P[c,f] Gr C: P[d,f]	Gr A + B: 1.6–5.1 Gr C: 6.9[e]	Gr A: 0.5–1.3 Gr B: 1.4–5.0[f] Gr C: 3.3[e,f]	Busulfan (myeloablative)	A: 0.1–0.2 B: 0.5–2.6[f] C: 2.5[f,g]	bluebird bio	– 5.3–18.2% HbA^{T87Q} in group A (18–27 months post–GT) – 51 and 28% Hb^{AT87Q} in group B (6 and 9 months post–infusion)	[112,116,120, 121,247]
NCT02906202 (HGB–207) III Global July 2016	Non-β^0/β^0 TDT: 23 recruiting	BB305 β^{A-T87Q}	G/P	7–13.6	2.4–4.0	Busulfan (myeloablative)	0.3–3.4	bluebird bio	Three patients treated (as of June 2017) and free of transfusion	[113, 114, 171, 173, 248]
NCT02906202 (HGB–212) III Global June 2017	β^0/β^0 TDT: 15 recruiting	BB305 β^{A-T87Q}	G/P	NA	NA	Busulfan (myeloablative)	NA	bluebird bio	NA	[115, 249]

[a]10 patients with TDT or SCD were expected; three TDT were included and one received back-up cells; two subjects (1003 and 1004) are described.
[b]The PB VCNs for two of the three SCD patients (0.3) was measured 3 and 6 months post-infusion and may increase.
[c]Plerixafor mobilization and apheresis used for collection of rescue cells only.
[d]Plerixafor mobilization and apheresis used for drug product and rescue cells.
[e]Median value for four patients.
[f]Revised manufacturing process of the drug product for patients in groups B and C.
[g]One patient only, 1 month post-infusion.
G=G-CSF; P=plerixafor; BM=bone marrow harvest; DP=drug product; PB=peripheral blood; Gr=Group; Aust=Australia; Thai=Thailand; NA=not available; GT=gene therapy

of patients in need. However, transduction efficiency was limited to ~10–20% of HSCs and HbA^{T87Q} levels only reached ~3 g/dL.

Worldwide Phase II trials. Because the chromatin insulator incorporated in the HPV569 LV decreased vector titers while contributing to HMGA2 activation by splicing interference [103], we decided to remove it from HPV569, also following the advice of the US and European regulatory agencies. The resulting LentiGlobin vector is referred to as BB305. In this LV, the cytomegalovirus promoter was used to drive vector transcription in packaging cells [96]. No other changes were made to the LentiGlobin vector. The titer of the new vector was three to four times higher than that of HPV569 and its $CD34^+$ transduction efficiency was two to three times greater. The two vectors had similar integration patterns in mouse cells, no risk of immortalization was detected in vitro or in vivo, and they gave similar levels of β^{AT87Q}-globin expression on a per-gene basis [96]. Based on these new preclinical results and the sustained transfusion independence observed in LG001's Patient 1003, the regulatory authorities gave their approval for three new clinical trials sponsored by bluebird bio: HGB-205 (NCT02151526) [110] in France; HGB-204 (NCT01745120 or "Northstar") [111] at multiple centers in the United States, Australia, and Thailand; and HGB-206 (NCT02140554) [112] at multiple centers in the United States (Table 1). The HGB-205 trial follows on the LG001 study and has included seven patients with TDT or SCD. The HGB-204 and HGB-206 studies focused on TDT (18 patients enrolled) and SCD (29 patients planned), respectively. All three protocols follow essentially the same procedure as for the LG001 study except that the BB305 vector, which is further purified by preparative chromatography and ultrafiltration, is used instead of its parent vector, HPV569.

By the end of 2017, both HGB-204 and HGB-205 have completed inclusion of TDT patients, and 22 TDT patients (aged 12–35 years) have been treated with a median of 26 months (range 15–42) after gene therapy [108]. Thirteen of these patients had non-β^0/β^0 genotypes (including nine β^E/β^0) and nine had a β^0/β^0 genotype ($n=8$) or equivalent in severity (homozygous IVS-1 nt110G>A; $n=1$). The injected doses of $CD34^+$ cells (obtained upon granulocyte colony-stimulating factor [G-CSF] + Plerixafor mobilization) ranged between 5.2 and 18.1×10^6 cells/kg. VCN ranged between 0.3 and 2.1 in drug products (i.e., transduced $CD34^+$ cell populations) and between 0.1 and 4.2 copies/diploid genome in patients' PBMCs. No replication-competent lentivirus has been detected and no safety issue was attributed to the LentiGlobin vector in either study. Serial monitoring of vector integration sites in blood samples has consistently shown polyclonal profiles of UIS without dominant clone. The median number of UIS was 1822 and 2364 per patient at 12 months after infusion for HGB-204 and HGB-205, respectively [108].

HbA^{T87Q} levels in patients with a non-β^0/β^0 genotype and PBMC VCN > 0.1 ranged from 3.4 to 10.0 g/dL at last measurement. These patients produced only up to 2.9 g/dL of endogenous HbE (15–21% of total Hb) and had insignificant HbF in the blood. All but one patient with a non-β^0/β^0 genotype ($n=9$ for HGB-204, and $n=3$ for HGB-205) stopped chronic RBC transfusions, with a total Hb of 10.5 (range 8.2–13.7) g/dL at the most recent study visit (12–36 months). Therefore, importantly, several patients showed near complete disease correction with Hb levels approaching or within normal range for their genders [108]. For patients treated with regular phlebotomies to help decrease iron accumulation, withdrawing 200 mL of blood each month, blood Hb levels have been stable despite a cumulative phlebotomy volume of >1 L per patient.

The impact of gene therapy on hemolysis and dyserythropoiesis was also assessed in patients enrolled in HGB-205. After having become transfusion independent after gene therapy, hemolysis first stabilized relative to pretransplantation levels (when patients were regularly transfused) and fully corrected in two patients. One patient has been able to stop all treatments for iron overload because the results of all biological and MRI studies have fully normalized. Plasma levels of two markers of ineffective erythropoiesis or erythroid expansion, the soluble transferrin receptor (sTFR) and erythroid protoporphyrin IX (EPP-IX), are within normal ranges for the three β^E/β^0 patients. In addition, plasma hepcidin/ferritin ratios have increased substantially over time.

Most of the eight patients with β^0/β^0 genotypes in the HGB-204 study continue to receive transfusions; however, there was a median 67% (range 7–100%) reduction in the annual number of transfusions and a 73% reduction (range 19–100%) in the annual transfusion volume compared with transfusion support in the 2 years before enrollment. Importantly, RBC transfusions were stopped in three such patients (two patients with β^0/β^0 genotypes in HGB-204 and the patient homozygous for the severe IVS1-110 mutation in HGB-205). The latter patient stopped RBC transfusions 3 months after gene therapy and had total Hb of 8.3 g/dL at last follow-up, of which 6.6 g/dL was HbA^{T87Q}.

Worldwide Phase III trials. On the basis of Phase II results, two pre-drug marketing Phase III trials have been initiated for TDT with the same LV (LentiGlobin BB305). These are multicenter, global (worldwide) studies sponsored by bluebird bio and were approved as "pivotal" and "confirmatory" by the US and European regulatory authorities, respectively (Table 1). HGB-207 ("NorthStar-2"; NCT02906202) [113,114] and HGB-212 ("NorthStar-3"; NCT03207009) [115]

are for non-β^0/β^0 and β^0/β^0 genotypes, respectively. Both trials are open and enrolling patients. HGB-207 will include 15 adult and adolescent patients together with eight pediatric patients; HGB-212 will include a mixture of 15 adult, adolescent, and pediatric patients. To increase $CD34^+$ transduction efficiency, two small proprietary molecules are utilized as transduction enhancers that are part of a refined manufacturing process.

Gene therapy of SCD

Proof-of-principle in a human patient. To date, three and nine patients with severe SCD (β^S/β^S or β^S/β^0) have been included in the HGB-205 and HGB-206 studies, respectively (Table 1). $CD34^+$ cells were harvested from bone marrow and injected at a dose of 1.6 to 5.6×10^6 cells/kg. Following the first seven patients included the HGB-206 study, changes to the study protocol have included manufacturing improvements, increasing the target busulfan area under the curve, introducing a minimum period of regular blood transfusions before stem cell collection, and including peripheral blood cell mobilization [116]. At the initial manufacturing process, the VCNs of the drug products were similar between the two studies, at 0.5–1.3. The VCN in the peripheral blood of patients included in the HGB-205 trial (0.3–2.3) was consistent to that in the drug products [117]. The first patient to be treated in the HGB-205 trial was a 13-year-old male with a β^S/β^S genotype, a history of numerous VOCs and ACS, and regular transfusions before treatment to keep HbS levels below 30% [117,118]. Two days after a full myeloablative dose of busulfan, he received 5.6×10^6 $CD34^+$ cells/kg, with a VCN of ~1. Fifteen months after treatment, the VCN in PBMCs cells was ~2 and the total Hb concentration was 11.8 g/dL, with 48% HbA^{T87Q} and 49% HbS [118]. Biological parameters improved, consistent with a clinical benefit. RBC transfusions were stopped 3 months after transplantation and no SCD-related complication occurred over the 15 months after gene therapy. Thirty months after treatment, the patient developed a VOC after an episode of acute gastroenteritis. His total Hb and HbA^{T87Q} levels were 12.4 and 6.1 g/dL respectively, and peripheral blood VCN has remained stable. Two other patients were included more recently. Gene transfer efficiency was lower than that reported for the first subject, but follow-up (3 and 6 months) remains limited for these patients, although clinical benefit has already been observed [117,119]. For the first seven patients included in HGB-206 (group A), the VCN in PBMCs was approximately one-fifth (range: 0.1–0.2) that in drug products (0.5–1.3), likely caused by non-myeloablative busulfan dosage [117,120]. At a median of 15 months (12–21 months) after product infusion, HbA^{T87Q} levels were between 0.4 and 2.4 g/dL [120]. Recently, two patients received drug products manufactured using a refined process (Table 1) [121]. A marked increase of peripheral blood VCN was observed 1–3 months after infusion (0.5–2.6), likely resulting from increases of transduction efficacies in the drug products (2.9–5.0).

Other trials

Two other Phase I/II clinical trials for the LV gene therapy of TDT have been initiated, one in New York (NCT01639690) and the other in Milan, Italy (NCT02453477), sponsored by the Memorial Sloan Kettering Cancer Center and the Italian charity organization Fondazione Telethon, respectively (Table 2). Because the LVs used in these trials express the wild-type human β-globin gene, no direct determination of LV-encoded therapeutic globin product is feasible. The New York protocol is different from the others because it includes a low-intensity conditioning regimen (8 mg/kg busulfan) rather than a fully myeloablative regimen [122,123]. The study was suspended to allow for testing of a revised LV [123]. The Italian trial includes myeloablative conditioning with thiotepa and treosulfan. Preliminary data were presented at the European Hematology Association and American Society for Hematology meetings in 2017 for three adults with a β^0/β^+ genotype and four children, two with β^0/β^+ and two with β^0/β^0 genotypes. All received 16 to 19.5×10^6 $CD34^+$ cells/kg previously transduced with the GLOBE LV [124–126]. VCN ranged from 0.7 to 1.5 in the drug products and reached 0.4–1.5 in PBMCs. Transfusion requirements decreased in all subjects except for one child with a β^0/β^0 genotype. Transfusion independence was reported for three children, including one with the β^0/β^0 genotype, but there had been only 8–13 months of follow-up after gene therapy by the time these findings were reported.

Two other vectors shown to be effective in preclinical studies are currently being evaluated in humans with SCD [87,127,128]. One contains a β-globin gene with three anti-sickling mutations (NCT02247843), which will be administered to six adults in a Phase I/II trial. The other contains the γ-globin gene (NCT02186418) and will be used in a Phase I/II trial. No results for these trials are yet available (Table 2).

Alternative, non-gene therapy approaches for the treatment of TDT are also under investigation by others, such as an activin receptor ligand trap or a Jak2 antagonist [129,130], and allogeneic transplantation with alternative donor sources, conditioning regimens, or GVHD prophylaxis [131]. In addition, gene editing by CRISPR/Cas9 is investigated at the preclinical stage [132].

Table 2. Human clinical trials to date for gene therapy of TDT or severe SCD with other LVs

Trial identifier Phase Location Start date	Condition Patients	Vector Gene	$CD34^+$ collection	Cell dose ($\times 10^6$/kg)	DP VCN	Conditioning	PB VCN	Sponsor	Comments	References
NCT01639690 I US July 2012	TDT: 4	TNS9.3.55 β^A-globin	G	8.3−12	0.2−0.4	Busulfan (nonmyeloablative for 3 patients)	0.02−0.08	Memorial Sloan Kettering Cancer Center	One patient with a significant decrease in transfusion requirement; this trial is currently suspended and has been amended for higher-intensity chemotherapy conditioning	[87,122,123,127,250]
NCT02453477 I/II Italy May 2015	β^0/β^0 and non-β^0/β^0 TDT: 10[a]	GLOBE β^A-globin	G/P	16−19.5	0.7−1.5	Thiotepa + threosulfan (myeloablative)	0.37−1.55	IRCCS San Raffaele	Reduction of transfusion requirements in 3/3 adults (16−22 months post-GT); transfusion independence in 3/4 pediatrics	[125,126,170,172,251]
NCT02186418 I/II US July 2014	SCD: 10	sGbG γ-globin	BM	NA	NA	Melphalan (myeloablative)	NA	Children's Hospital Medical Center, Cincinnati	NA	[87,127,128,252]
NCT02247843 I US August 2014	SCD: 6	βAS3-FB β^{AS3}-globin[b]	BM	NA	NA	Busulfan (myeloablative)	NA	University of California Children's Hospital, Los Angeles	NA	[87,127,128,253]

[a]As of August 2017, seven out of 10 patients have been treated.
[b]Triple-mutant β-globin (G16D, E22A, and T87Q).
G=G-CSF; P=plerixafor; BM=bone marrow harvest; DP=drug product; PB=peripheral blood; NA=not available; GT=gene therapy

Critical factors affecting efficacy

For TDT

In β-thal, unbound β-hemoglobin chains precipitate at the cell membrane, resulting in apoptosis of erythroid precursors and destruction of mature RBCs [133]. Gene therapy thus aims not only to provide adequate Hb levels for its oxygen-carrying properties, but also to increase β-globin concentration sufficiently within RBCs to ensure the binding of unpaired α-hemoglobin, thereby protecting erythroid cells from premature elimination. A key endpoint of efficacy after gene therapy for TDT is sustained conversion to transfusion independence, usually defined as Hb levels maintained in the absence of RBC transfusion >7–8 g/dL with no major clinical symptoms of anemia. Among transfusion-independent patients, however, clinical tolerance and, to a lesser degree, Hb levels and the degree of correction of dyserythropoiesis differentiate full correction (asymptomatic; Hb levels within normal ranges for gender and full correction of dyserythropoiesis), thalassemia minor or trait (asymptomatic or mildly symptomatic; mild anemia above 9 g/dL Hb and/or microcytosis; mild evidence of dyserythopoiesis), and thalassemia intermedia (symptomatic; microcytic anemia; evidence of dyserythropopiesis and iron overload). Patients with β-thal intermedia may experience increased morbidity, mortality, and compromised quality of life with age resulting from silent brain infarcts, thrombosis and pulmonary hypertension, hepatocellular carcinoma due to iron overload, and leg ulcers [134,135]. Even in β-thal minor, a recent study on a large cohort of individuals suggested a mildly increased risk of cirrhosis, kidney disorders, cholelithiasis, and mood disorders [136]. The goal of gene therapy for TDT thus aims first at achieving sustained transfusion independence, then converting patients from β-thal intermedia to β-thal minor status, and ultimately approaching full disease correction.

Results from our Phase II trials worldwide with 22 treated TDT patients [108] suggest that our current LentiGlobin vector (BB305) and protocol are uniformly therapeutic in patients with β^{E}/β^{0}-thal, who represent approximately half the worldwide cases of TDT. Virtually all such patients converted to sustained transfusion independence, reaching β-thal minor status or even approaching full disease correction. In several such patients, Hb levels reached or approached normal ranges for healthy individuals, thereby correcting dyserythropoiesis. One patient also normalized iron overload and was able to stop both iron chelation and therapeutic phlebotomies. In contrast, for most individuals with a β^{0}/β^{0} genotype or equivalent, RBC transfusion support after treatment was reduced but not eliminated, although three such patients have stopped RBC transfusions. One hopes that further improvements in our ongoing Phase III trials will be consistently therapeutic even for patients with a β^{0}/β^{0} genotype, converting them not only to transfusion independence but also to β-thal minor status or full correction.

Impact and optimization of chimerism levels with corrected cells. As anticipated from preclinical mouse studies and initial clinical results in our initial Phase I/II trial, data from our worldwide Phase II trials indicate that blood HbA^{T87Q} levels correlate with VCN values in PBMCs at 6 months, as shown from regression analysis. This is the single most important predictive parameter for clinical efficacy in the peripheral blood after gene therapy [108]. VCN in PBMCs is itself related to VCN in the drug product (transduced $CD34^{+}$ cells), $CD34^{+}$ cell dose administered, and the degree of myeloablation after conditioning.

After allogeneic transplantation, most of RBCs in TDT patients with moderate but persistent mixed chimerism (20–40% donor-derived nucleated cells) are of donor origin [137], consistent with the preferential survival (selection) of normal erythroid cells and the high rates of erythroid precursor apoptosis and mature RBC hemolysis in TDT [138]. Those patients no longer require RBC support and their Hb levels are 8–11 g/dL [139,140]. Therefore, improvements in quality of life through the acquisition of transfusion independence can be achieved by ensuring mixed chimerism of a sufficiently high degree with genetically modified HSCs. In the setting of gene therapy, in vivo VCN depends on both transduction efficiency and donor/recipient chimerism, which cannot be measured in the setting of human autologous transplantation.

The cell transduction efficiency of LVs is difficult to control and predict, particularly for HSCs, and is highly dependent on patient samples [120,141–143]. Furthermore, no definitive correlation between VCN in surrogate assays (such as mouse transplantation experiments, long-term culture-initiating cells, or colony-forming cells) and VCN in patients' PBMCs has been demonstrated for the evaluation of HSC transduction efficiency [120]. Many efforts have been made to increase LV transduction efficiency in hematopoietic $CD34^{+}$ cells as a whole using mostly LV pseudotyped with the vesicular stomatitis virus envelope glycoprotein [144]. Various molecules, such as cationic additives, which neutralize membrane charges and promote interactions between cells and viruses, have been shown to enhance $CD34^{+}$ cell transduction efficiency [145–147]. Certain of these molecules are used in the preparation of gene therapy products for clinical studies [103,104,118].

The optimization of vector backbones to increase vector titers [96] and improvements in vector

purification procedures [148] have also increased mean VCN in patients' cells, in vitro and in vivo [118,149–151]. Compounds that inhibit post-entry trafficking from the plasma membrane to the nucleus, such as inhibitors of proteasome [152,153], cyclin-dependent kinase p21 [154], or mTOR [155] and various other molecules [156], may be used in the future if preclinical studies demonstrate a preservation of primitive HSCs [157]. A key difficulty in the clinical use of these compounds is that it is unclear whether the improvement observed in vitro and in mouse studies will translate into an optimization of transduction efficacy in human cells in the long term and the preservation of HSCs. Preclinical studies in nonhuman primates (NHPs) can effectively evaluate and compare the fate and transduction efficacy of HSCs, but their cost, the complexity of post-transplantation supportive care for animals receiving ablative conditioning, and ethical concerns have greatly restricted their use. Furthermore, HIV-derived LVs have the inherent disadvantage of low transduction frequencies in NHP cells due in part to host restriction by TRIM5 [158]. Finally, differences in myeloablative conditioning regimen (total body irradiation) relative to human protocols (alkylating agents) may make it difficult to compare the fates of human and simian HSCs. Hematopoietic cell transduction protocols therefore tend to follow a standard ex vivo procedure involving $CD34^+$ cell sorting, activation, and transduction in the presence of regular activating cytokines for a limited period of time (2–4 days) and infusion by intravenous injection [103,104,150,159–161]. Protocols may be optimized by shortening the ex vivo culture time to maintain HSC function [162,163], sorting and transducing more primitive cells [164,165] to reduce the amount of vector and to decrease the number of potentially dangerous insertional mutations, refining culture and cytokine concentrations to accelerate platelet and granulocyte recovery [166], incorporating molecules to expand [167,168] or transduce [169] HSCs, and intraosseous infusion of the modified HSCs to favor engraftment and to reduce cell loss [170]. Two modifications are already being evaluated in clinical trials on TDT patients: the incorporation of transduction enhancers in bluebird bio's Phase III HGB-207 ("NorthStar-2"; NCT02906202) and HGB-212 ("NorthStar-3"; NCT03207009) trials [113,171] and intraosseous infusion at the San Raffaele IRCCS in a Phase I/II study evaluating the efficacy and safety of the GLOBE LV in child and adult subjects [170,172].

Encouraging data released by the sponsor of the HGB-207 study suggest that the median VCN in the drug products is higher than achieved in previous studies and that the in vivo VCN is at least as good as that in patients achieving transfusion independence in the HGB-204 clinical trial [173]. It is anticipated that the higher VCN obtained in the drug product will make it easier to achieve transfusion independence in the patients, especially for the β^0/β^0 genotypes (HGB-212).

Target HbA^{T87Q} and total Hb levels for clinical efficacy. In the first TDT patient to become transfusion independent after lentiviral gene therapy in our initial Phase I/II clinical trial [103], 10–20% of the bone marrow erythroid progenitors were genetically modified. Based on the observation of patients with mixed chimerism after allogeneic transplantation [139,140], the proportion of corrected RBCs was thus ~70% in this treated individual. The therapeutic β^{AT87Q}-globin contributed ~10 pg to mean corpuscular hemoglobin (MCH) levels. In heterozygous individuals with β-thal minor (β^A/β^0), who are generally considered to be healthy despite mild hypochromic microcytic anemia (Hb ≥10.0–11.0 g/dL depending on gender), MCH is ~20 pg [174,175]. Therefore, in the RBCs of the first TDT patient successfully treated by gene therapy [103], the ratio of therapeutic β^{AT87Q}-globin output to mean normal endogenous β^A-globin output on a per-gene basis was ~(10/20)/0.7 = 70%, which is in the range of measurements recorded for human cells transduced with a similar LV and injected into immunodeficient mice [141]. We can therefore estimate the minimum LV copy number per nucleated cell (CPC) required for a β^0/β^0 TDT patient to become transfusion independent at between 3 CPC in 20–40% of nucleated cells and 1–2 CPC in 100% of nucleated cells. The target β-globin level and CPC vary slightly with the patient's residual β-globin expression: absent (β^0) or present at suboptimal levels (β^+). They also depend on genetic modulators such as α-thal [176,177] or the ability to produce substantial amounts of γ-globin after birth [178,179], both of which have been shown to reduce disease severity.

These estimates were confirmed in our worldwide Phase II trials [108]. In addition, Hb levels drove the correction of biological signs of dyserythropoiesis [108]. Hb levels correlated negatively with both sTFR and EPP-IX in RBCs. Hb levels were correlated with hepcidin/ferritin ratios and to a lesser extent to hepcidin. Beyond 9.5 g/dL of Hb, erythroid mass was reduced and peripheral iron control was restored by hepcidin. Below 8.5 g/dL, ineffective erythropoiesis was maintained and hepcidin was repressed.

For SCD

In SCD, the substitution of valine for glutamic acid at position 6 of the β-globin chain [180] is responsible for deoxygenation-induced HbS ($\alpha_2\beta^S{}_2$) polymerization, the primary molecular event that drives RBC sickling and hemolysis, increases in blood viscosity, VOC and ACS, strokes, and multiple-organ damage [181]. The

clinical severity of SCD depends on the proportion of HbA ($\alpha_2\beta^A{}_2$), HbF ($\alpha_2\beta_2$), or HbA2 ($\alpha_2\delta_2$) [182]. Intracellular HbS polymerization results from the interaction of the mutated valine in a tetramer complex with an acceptor hydrophobic pocket (β^{F85} and β^{L88}) on a close tetramer [183]. The β^{87} residue modulates HbS tetramer interaction within the acceptor hydrophobic pocket [100,184]. This residue is a threonine in the β^S- and β^A-globin chains (T87), but a glutamine in the γ- and δ-globin chains (Q87), and it plays an essential role in excluding γ and δ hemoglobin from HbS polymers [185,186]. HbF, HbA2, and the hybrid tetramers ($\alpha_2\beta^S\gamma$ and $\alpha_2\beta^S\delta$) cannot be incorporated into the polymer [187–189]. We thus implanted the TQ87 mutation into an optimized β-globin expression cassette [102] suitable for LV transfer [91,103] as a means of producing large amounts of anti-sickling Hb in adult RBCs. Biochemical assays confirmed that the mutant β^{AT87Q}-globin chain inhibited HbS polymerization as efficiently as the γ-chain, correcting SCD in two transgenic mouse models [91]. The oxygen affinity of the resulting tetramer of human Hb ($\alpha_2\beta^{AT87Q}{}_2$) was in the range of that of HbA, an advantage over anti-sickling HbF, which has a substantially higher oxygen affinity [190]. The T87Q mutation is also a valuable marker for evaluating therapeutic gene expression in RBCs because β^{AT87Q} can be distinguished from β^A- and γ-globin chains by reverse-phase high-performance liquid chromatography [106]. This approach is particularly useful for detecting the transgenic protein in β^+ thalassemia patients or in β^0 individual transfused with normal RBCs. The same β^{AT87Q}-globin-expressing LV can therefore be used to treat both diseases.

Support for a therapeutic role of HbF has grown from observations in Indian and Saudi Arabian homozygous SS subjects with all the symptoms of SCD but a milder clinical course, with HbF levels of ~25% [191–193]. This situation is best illustrated by subjects with compound heterozygosity for HbS and gene deletion, with an inherited persistence of HbF (S-HPFH), a condition with very few symptoms [194]. In these patients with high HbF levels (typically 30% [195]), the benign clinical course of the disease has been attributed to the pancellular distribution of HbF, as opposed to the heterocellular distribution pattern seen in other patients with high HbF levels but with more serious phenotypes [196]. In individuals with S-HPFH, each RBC contains 25–30% HbF and is less likely to undergo sickling than the RBCs of patients in which HbF levels are low in some RBCs, contributing to the disease [197]. This conclusion is supported by mouse studies showing that complete RBC replacement is required for full correction [198]. Similarly, chronic blood transfusion to maintain a target HbS concentration of 30% or less leads to fewer complications and a better quality of life in children with SCD, but patients are not disease free [199,200]. Therefore, patients treated with gene therapy will experience an improvement if anti-sickling hemoglobin levels of at least 25–30% can be achieved and this improvement is stronger if the anti-sickling hemoglobin displays a pancellular distribution. Levels of at least 10 pg of anti-sickling Hb per cell should be protective [201]. As for TDT patients, SCD subjects undergoing transplantation and displaying mixed chimerism with >50% donor nucleated cells have donor RBC chimerism levels of>90% [202,203], consistent with an SCD-associated impairment of erythropoiesis and/or poor SS RBC survival [204]. SCD symptoms were alleviated in one subject with a mixed chimerism level as low as 11% [205]. Recent data from the National Institutes of Health on three SCD patients with mixed chimerism after allogeneic transplantation with HLA-compatible β^A/β^S donor marrow cells suggested that 20% donor cells are sufficient to alleviate most SCD manifestations [206]. However, the minimal level of mixed chimerism required depends on SS RBC survival [206]. Furthermore, the presence of noncorrected erythroid cells and the effect of residual hemolysis on long-term outcome require further evaluation.

Based on the above calculations, we believe that the delivery of VCN ≥1 per erythroid cell should ensure that anti-sickling Hb levels are high enough to cure most patients with SCD. The proportion of genetically modified nucleated cells should be at least 20%. A recent re-examination of health outcomes in subject with sickle cell trait (SCT: heterozygote β^A/β^S with ≥60% HbA) indicated that SCT was associated with certain adverse outcomes [207,208]. Therefore, as for TDT, future recommendations for the target VCN may need to be adjusted upward.

Safety

Although no major adverse event has been reported with LVs to date [209], safety concerns remain due to their potential to cause gene disruption and aberrant splicing events [210,211]. As stated above, clonal expansion due to vector integration has been documented in one patient with TDT treated by LV gene therapy [103]. It remains unclear whether the transduction process is homogeneous in hematopoietic cell subsets and whether the distribution of inserted LVs in HSCs is consistent with the values calculated from the mean VCN measured in $CD34^+$ cells and based on Poisson statistics [212]. The transduction efficiencies for various hematopoietic cell fractions ($CD34^+CD38^-$ and $CD34^+CD38^+$) enriched in stem or progenitor cells were shown to be higher for the most primitive cells [157,213]. In the SCD subject successfully treated by gene therapy, the peripheral blood cell subset contained

two to three vector copies per cell, whereas the VCN (≈1) was different in the bulk $CD34^+$ cells before infusion [118]. Therefore, increasing VCN through the addition of a particular molecule may increase the transduction of a small subset of cells [214] and increase the risk of cell transformation. Careful long-term evaluation of patients receiving transduced cells with a high LV copy number per cell is required before any definitive conclusions on the risks associated with this procedure can be drawn. One solution to this problem would be the transduction of cells at a relatively low mean VCN (between 0.5 and 1) and the selection of transduced HSCs from the total cell population before infusion [214]. This would have the additional advantage of preventing possible gene silencing and the age-dependent extinction of transgene expression [215]. However, this advantage is counterbalanced by technical and medical issues such as the longer time required for cell selection, which is undesirable because increasing culture time leads to a loss of engraftment potential and a decrease in clonal diversity [216,217]. Furthermore, the decrease in total cell number due to ex vivo selection may make it necessary to increase the number of cells used for transplantation by either improving $CD34^+$ cell harvesting or using effective ex vivo HSC expansion strategies [168]. The homogeneous engraftment of genetically modified cells depends on a rich and diverse HSC pool. It has been suggested that optimized conditions for the culture of hematopoietic cells may prevent, or at least delay, the outgrowth of cells with insertional mutations through their competitive suppression due to an excess of intact HSCs [218].

Other transplantation outcomes, including neutrophil and platelet recovery, growth factor requirement, time spent in hospital [219–222], and donor chimerism [223,224], are closely linked to the number of hematopoietic stem/progenitor cells ($CD34^+$) infused. Neutrophil and platelet recovery rates affect survival. Optimal chimerism is a prerequisite for complete disease correction. In addition, a backup of unmodified $CD34^+$ cells is stored, for rescue in cases of engraftment failure. It is therefore important to collect and transplant as many hematopoietic stem/progenitor cells as possible. Mobilized peripheral blood cells are the preferred source of HSCs because of the large number of $CD34^+$ cells obtained, the minimally invasive nature of the procedure, and the faster hematopoietic recovery than for patients receiving bone marrow cells [225]. However, the use of cytokines such as G-CSF which increase the number of white blood cells and their adhesion to the endothelium [226,227], have detrimental effects in patients with SCD, promoting acute complications [228–232]. Autologous hematopoietic $CD34^+$ cells have therefore to date been obtained by bone marrow harvest in subjects with SCD [87,149,233]. In splenectomized TDT patients, G-CSF-induced hyperleukocytosis is a dose-limiting factor, indirectly resulting in poor $CD34^+$ cell yields [234]. Plerixafor and G-CSF have been shown to display strong synergy [235] and this combination is thus preferred as a mobilization procedure for β-thal patients [87,236]. Plerixafor does not induce leukocytosis to the same extent as G-CSF. In an SCD mouse model, plerixafor has been shown to mobilize HSC subsets effectively without neutrophil or endothelial activation and with lower total white blood cells and neutrophil counts than in G-CSF-treated mice [237]. It is currently being tested in humans with severe SCD [112,238], including several patients currently enrolled in the HGB-206 trial [112]. $CD34^+$ cell yields are lower with bone marrow than with mobilized peripheral blood cells. This harvesting procedure may enhance hematopoietic (stem) cell recovery, resulting in higher levels of donor/recipient cell chimerism in patients with SCD.

Factors affecting patients with Hb disorders, such as iron overload and marrow dysfunction, have been shown to affect donor cell yield. Iron overload is inversely correlated with HSC mobilization rate and time to hematopoietic recovery [239,240]. Bone marrow hyperplasia limits the effect of conditioning and increases the risk of graft rejection after allogeneic transplantation [241,242]. Anemia with concomitant high levels of erythropoietin secretion may be associated with the reprograming of $CD34^+$ cells into double-positive $CD34^+CD36^+$ cells in vivo [243,244] and may affect the number of HSCs in the $CD34^+$ cell pool. It is therefore important for patients to undergo a period of hypertransfusion (or exchange transfusion for SCD) and concomitant appropriate iron chelation therapy before HSC harvesting and infusion of the drug product. Furthermore, transduced HSCs from patients with Hb disorders have no selective advantage relative to non-corrected cells and cells grown ex vivo probably have a competitive disadvantage relative to unmanipulated recipient cells. It is thus essential to use a full dose of the myeloablative agent as a pre-transplantation conditioning regimen. In the HGB-205 study, busulfan is administered intravenously at an initial dose of 3.2 mg/kg/day for 4 consecutive days [103,118]. Plasma busulfan concentration is meticulously monitored by daily pharmacokinetics analyses [245] and the dose is adjusted according to the target value. In the original protocol of the HGB-205 study, the target myeloablative dose of busulfan for a daily dosing regimen ranged between 3200 and 4400 μmol/L/min/day. This dose has been modified to 4000–5200 μmol/L/min/day on the basis of the clinical experience of the principal investigator, who has found that higher levels of busulfan exposure improve efficacy outcomes [118].

Conclusions

The potential of gene therapy to address the root cause of inherited disorders, including the β-hemoglobinopathies, rather than simply alleviating their symptoms and to provide long-term therapeutic effects after a single administration may bring hope for a curative treatment for many affected patients. Gene therapy by gene addition has made great strides forward since the discovery of the regulatory elements that control β-globin transcription, the advent of LVs, the development of preclinical globin vectors, and the proof-of-principle of efficacy in mouse models [103,118]. On a path to market approval, the LentiGlobin vector BB305, now in a global Phase III trial, has resulted in transfusion independence in several patients with TDT and clinical benefit in a patient with SCD. In particular, this vector and current protocol appear consistently therapeutic for TDT patients with β^{E}/β^{0}, the genotype responsible for approximately half of all TDT cases. Key achievements and remaining limitations and room for improvement are summarized in Figure 1. The implementation of gene therapy in clinical practice for TDT and SCD will

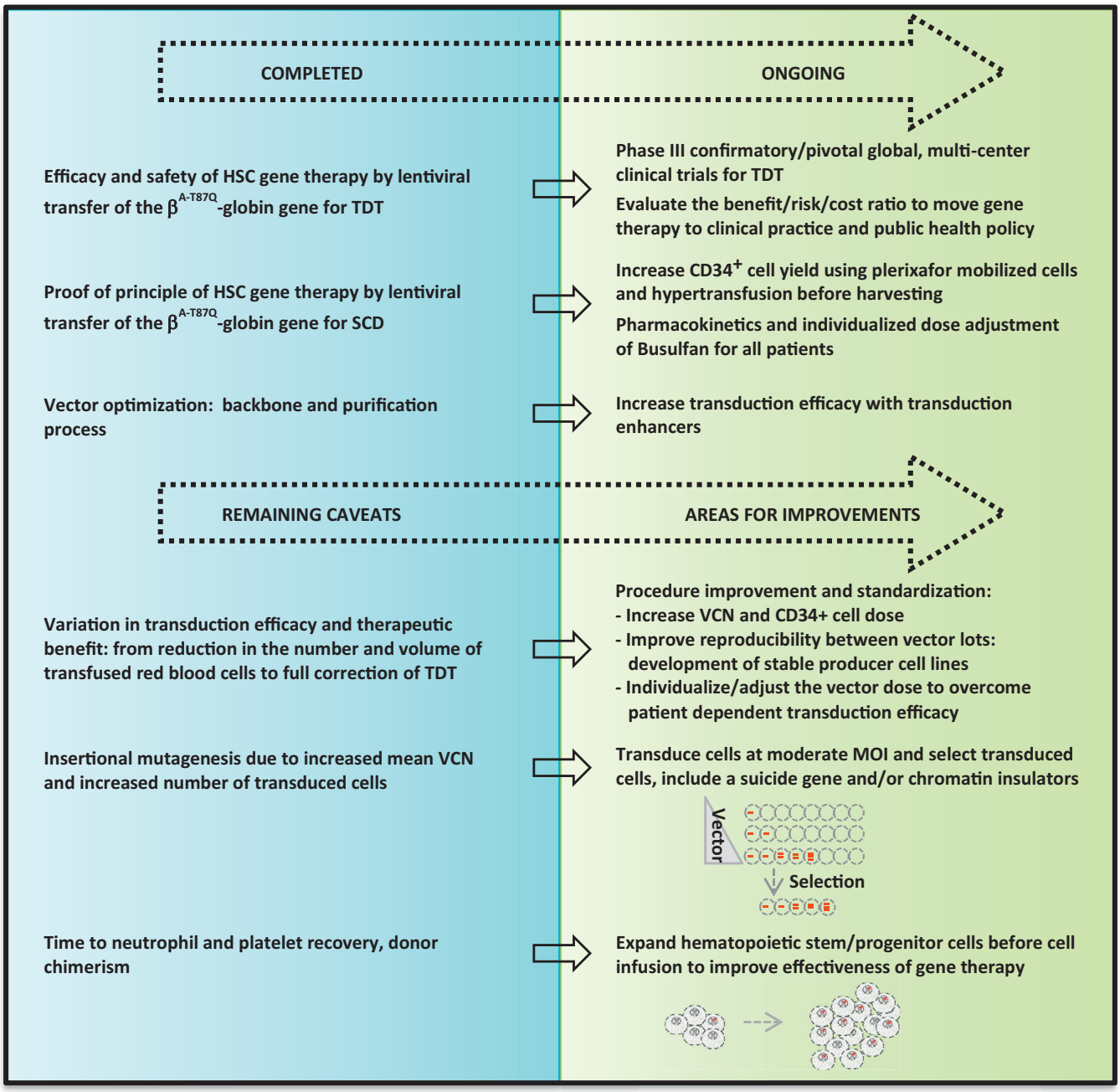

Figure 1. Gene therapy for Hb disorders: key achievements, ongoing direction, and room for improvement.

ultimately depend on long-term benefit/risk/cost ratios, which will be carefully evaluated over the next few years.

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Conflict of interest disclosure

PL has financial relationships with bluebird bio, Inc. The remaining authors declare no competing financial interests.

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