Gene replacement therapy for hemoglobinopathies: clinical benefit and challenges for widespread utilization

Marina Cavazzana, Annarita Miccio, Isabelle Andre-Schmutz & Fulvio Mavilio

β-thalassemia and sickle cell disease (SCD), the most common genetic disorders worldwide, are caused by mutations affecting quantitatively or qualitatively hemoglobin β-chain production. With the advent of β-globin expressing lentiviral vectors (LVs), transplantation of autologous, genetically modified hematopoietic stem cells (HSCs) holds the promise of eliminating the morbidity and mortality associated with allogenic HSC transplantation and circumventing the need for suitable donors. LV-based gene addition strategies have shown safety and efficacy in both β-thalassemia and SCD patients. Despite these promising results, gene therapy still requires significant development to become standard clinical practice. Here, we discuss several issues that limit the efficacy and the utilization of LV-based gene therapy approaches for β-hemoglobinopathies, such as the source and the quality of HSCs, the choice of the optimal conditioning regimen, the cell manufacturing process and the regulatory framework.

Submitted: 4 September 2018 ▶ Published: 15 October 2018

INTRODUCTION

Since the first successful gene therapy for X-linked severe combined immunodeficiency (X-SCID) in 2000, several clinical trials have confirmed the potential of a viral vector-mediated gene addition approach to the therapy of blood monogenic diseases [1–9]. While the X-SCID clinical trial provided the first proof of concept for this
strategy, it also showed that the use of first-generation gamma-retroviral vectors for gene transfer is associated to a very high risk of insertional mutagenesis [10]. This risk has been further confirmed by the occurrence of acute T-cell lymphoblastic leukemia and myelodysplastic syndromes in all clinical trials using the same type of vectors, i.e., for Wiskott–Aldrich syndrome (WAS) and chronic granulomatous diseases (CGD) [11,12]. The only, yet poorly explained, exception is gene therapy for adenosine deaminase deficiency, which showed no adverse consequences in all treated patients [5] and in 2016 became the first ex vivo gene therapy to be commercialized under the name of Strimvelis®.

The use of HIV-derived self-inactivated (SIN) retroviral vectors, first tested in a gene therapy trial for X-linked adrenoleukodystrophy (ALD) in 2005, has significantly reduced or abolished the risk of insertional mutagenesis in all indications tested. Although long-term follow-up studies are still required to rule out such risk, LVs allowed the development of gene therapy for more complex diseases such as β-hemoglobinopathies [8,9].

GENE THERAPY FOR HEMOGLOBINOPATHIES

Thalassemia and sickle cell disease (SCD) are the most frequent monogenic diseases worldwide, with approximately 5–7% of the world population carrying a hemoglobin disorder trait. Two major factors explain the prevalence/incidence and geographical distribution of these diseases, i.e., the endemicity of malaria and the forced population migrations from sub-Saharan Africa to the USA, Europe and the Arabic peninsula. In the USA, one every 67 newborns is a carrier of the sickling βS allele and one every 1,941 has SCD; in France, one every 3,000 newborns is affected by SCD, resulting in 385 new cases per year. To date, no African country has implemented a national screening program and no patient registry exists; the incidence of SCD in sub-Saharan Africa is estimated at 230,000 new cases per year, which corresponds to 75% of all births with SCD worldwide [13,14].

Gene therapy for hemoglobinopathies became possible with the introduction of the SIN lentiviral vectors (LVs), with their increased capacity to accommodate large transcriptional units as well as their higher transduction efficiency in long-term repopulating hematopoietic stem cells (HSCs). A major breakthrough occurred with the development of the first LV that featured an optimized β-globin gene under the control of the β-globin promoter, 3’ enhancer and the DNase I hypersensitive sites 2, 3 and 4 (HS2, HS3 and HS4) elements of the β-globin locus-control region (LCR). The prototype vectors (TNS9 and HPV569) expressed β-globin at relatively high levels and proved their therapeutic efficacy in correcting two murine models of β-thalassemia [15] and SCD [16]. Vectors approved for clinical trials are mostly derived from these prototypes (TNS9.3.55 and BB305, respectively), or from the GLOBE vector, containing only HS2 and HS3 elements (Table 1).

 Completed or currently ongoing clinical trials of gene therapy for hemoglobinopathies are listed in Table 1. The first patient affected by transfusion-dependent...
HbE/β-thalassemia was treated in June 2007 with autologous bone marrow (BM) HSCs transduced by a SIN LV expressing the βT87Q globin variant [17]. In this genetic background, the HbE variant is produced at low levels due to a splicing abnormality caused by the mutation, mimicking a mild β+-thalassemia allele and reducing the overall requirement for therapeutic β-globin synthesis. The patient received myeloablative conditioning based on the exclusively use of Busulfan followed by the infusion of 3.9 × 10^6 total CD34+ hematopoietic/stem progenitor cells (HSPCs) per kg of body weight. In the year that followed the infusion of the gene-modified cells the patient underwent a gradual increase of the proportion of gene-corrected cells up to 10–20%. Consequently, the patient became transfusion-independent with stable Hb levels reaching 8.5–9 g/dl 1 year after gene therapy. The transgene product (HbAT87Q), the HbE allele and a reactivation of fetal hemoglobin (HbF) synthesis contributed in almost equal proportion to the total circulating hemoglobin [17]. The patient maintained stable levels of therapeutic Hb for more than 9 years and is currently requiring only occasional transfusions. This pilot study provided the proof of concept that gene addition can benefit patients affected by β-hemoglobinopathies under appropriate conditions. It also showed that the number of gene-corrected HSCs and the vector titer and infectivity were two crucial parameters requiring improvement.

Two subsequent clinical trials, based on a modified version of the vector called BB305, addressed transfusion-dependent β-thalassemia. These were the multicenter Northstar HGB204 and the single-center HGB205 study, which started in 2013 and are ongoing in USA, Australia, Thailand and France. These studies are based on transplantation of autologous HSCs mobilized with a combination of G-CSF and Plerixafor, a bicyclam molecule that mobilizes HSPCs by antagonizing the binding of stromal cell derived factor-1 (SDF-1) to the chemokine CXC receptor-4 (CXCR4). The HGB205 trial uses an optimization of the HSC mobilization procedure, which is performed after 3 months of a hyper-transfusion regimen aimed at suppressing BM erythroid hyperplasia. As recently published [8], 12 patients with genotypes different from β0/β0 and 3 out of 9 β0/β0 patients have discontinued transfusions and remained stably transfusion-independent, while 6 β0/β0 patients decreased their transfusion requirement by an average 73% [8]. The level of transgene expression required to normalize Hb levels in β0/β0 patients is much higher than for other genotypes, partially explaining the difference in success rate in these patients. In both studies, gene therapy with the BB305 product was well tolerated with no gene transfer-related severe adverse event and evidence of polyclonal reconstitution without clonal dominance. Two Phase 3 international multicenter clinical trials based on BB305 product are currently ongoing for β-thalassemia patients with or without β0/β0 genotype (HGB212, HGB207) (Table 1).

A Phase 1/2 clinical trial also addressing transfusion-dependent β-thalassemia started in Italy in 2015 (NCT02453477) [18,19].
<table>
<thead>
<tr>
<th>NCT number (other name)</th>
<th>Phase</th>
<th>Indication (number of patients; age)</th>
<th>Vector/ transgene</th>
<th>HSC source</th>
<th>Conditioning</th>
<th>Sponsor</th>
<th>Location</th>
<th>Start of the study</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>sLG001</td>
<td>1/2</td>
<td>β-Thal major and SCD 5; β-Thal + SCD 5; 5–35 yrs</td>
<td>HPV569/βA-T87Q globin</td>
<td>BM CD34+ cells</td>
<td>Busulfan</td>
<td>Bluebird Bio</td>
<td>Paris, France</td>
<td>September 2006</td>
<td>Completed</td>
</tr>
<tr>
<td>NCT01639690</td>
<td>1</td>
<td>β-Thal major 10; &gt;18 yrs</td>
<td>TN59.3.55/β-globin</td>
<td>G-CSF-mobilized CD34+ cells</td>
<td>Busulfan (8 mg/kg [RIC] for 3 patients, 14 mg/kg for 1 patient)</td>
<td>Memorial Sloan Kettering Cancer Center</td>
<td>New York, NY, USA</td>
<td>July 2012</td>
<td>Active, not recruiting</td>
</tr>
<tr>
<td>NCT02453477</td>
<td>1/2</td>
<td>β-Thal 10; 3–64 yrs</td>
<td>GLOBE/β-globin</td>
<td>G-CSF+Plerixafor-mobilized CD34+ cells</td>
<td>Treosulfan and thiopeta</td>
<td>IRCCS San Raffaele</td>
<td>Milan, Italy</td>
<td>May 2015</td>
<td>Active, not recruiting</td>
</tr>
<tr>
<td>NCT02151526 (HGB-205)</td>
<td>1/2</td>
<td>β-Thal major and SCD 7; 3–35 yrs</td>
<td>BB305/βA-T87Q-globin</td>
<td>G-CSF+Plerixafor-mobilized CD34+ cells</td>
<td>Busulfan</td>
<td>Bluebird Bio</td>
<td>Paris, France</td>
<td>July 2013</td>
<td>Active, not recruiting</td>
</tr>
<tr>
<td>NCT01745120 (HGB-206)</td>
<td>1/2</td>
<td>β-Thal major 18; 12–35 yrs</td>
<td>BB305/βA-T87Q-globin</td>
<td>G-CSF+Plerixafor-mobilized CD34+ cells</td>
<td>Busulfan</td>
<td>Bluebird Bio</td>
<td>Oaklands, CA; Chicago, IL Philadelphia, PA, USA; Sydney, Australia; Bangkok, Thailand</td>
<td>August 2013</td>
<td>Completed</td>
</tr>
<tr>
<td>NCT02186418</td>
<td>1/2</td>
<td>SCD 10; 18–35 yrs</td>
<td>Gamma-globin</td>
<td>BM CD34+ cells</td>
<td>n/a</td>
<td>Children's Hospital Medical Center, Cincinnati</td>
<td>Cincinnati, OH, USA; Kingston, Jamaica</td>
<td>July 2014</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02247843</td>
<td>1</td>
<td>SCD 6; &gt;18yrs</td>
<td>βAS3-FB/βAS3 globin</td>
<td>BM CD34+ cells</td>
<td>Busulfan</td>
<td>Donald B. Kohn, California Institute for Regenerative Medicine, University of Southern California</td>
<td>Los Angeles, CA, USA</td>
<td>July 2014</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02140554 (HGB-206)</td>
<td>1</td>
<td>SCD 29; &gt;18yrs</td>
<td>BB305/βA-T87Q-globin</td>
<td>Plerixafor-mobilized or BM CD34+ cells</td>
<td>Busulfan</td>
<td>Bluebird Bio</td>
<td>Oakland CA, Chicago IL, Bethesda MD, New York NY, Philadelphia PA, Charleston SC, USA</td>
<td>August 2014</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02906202 (HGB-207)</td>
<td>3</td>
<td>β-Thal with transfusion dependence and no β0/β0 genotype 23; &lt;50 yrs</td>
<td>BB305/βA-T87Q-globin</td>
<td>G-CSF+Plerixafor-mobilized CD34+ cells</td>
<td>Busulfan</td>
<td>Bluebird Bio</td>
<td>Oakland CA; Chicago IL, Philadelphia PA, USA; Marseille, France; Hannover, Germany; Thessalonik, Greece; Rome, Italy; London, UK; Bangkok, Thailand</td>
<td>July 2016</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT03207009 (HGB-212)</td>
<td>3</td>
<td>β-Thal with 80/080 genotype 15; &lt;50 yrs</td>
<td>BB305/βA-T87Q-globin</td>
<td>G-CSF+Plerixafor-mobilized CD34+ cells</td>
<td>Busulfan</td>
<td>Bluebird Bio</td>
<td>Oaklands, CA, Chicago IL, Philadelphia PA, USA; Marseille, France; Hannover, Heidelberg, Germany; Thessalonik, Greece; Rome, Italy; London, UK</td>
<td>June 2017</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT03282656</td>
<td>1</td>
<td>SCD 7; 3–40 yrs</td>
<td>LV expressing an shRNA targeting BCL11A</td>
<td>BM CD34+ cells</td>
<td>Busulfan</td>
<td>David Williams, Boston Children's Hospital</td>
<td>Boston, MA, USA</td>
<td>February 2018</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT03351829</td>
<td>1/2</td>
<td>β-Thal 20; 4–70 yrs</td>
<td>SIN LV-n/a</td>
<td>n/a</td>
<td>Bluebird Bio</td>
<td>Children's Hospital Medical Center, Cincinnati</td>
<td>April 2019</td>
<td>Not yet recruiting</td>
<td></td>
</tr>
</tbody>
</table>

β-Thal: β-Thalassemia; LV: Lentiviral vector; n/a: Not available; RIC: Reduced-intensity conditioning; SCD: Sickle cell disease; SIN: Self-inactivating; yrs: Years.
The study used autologous G-CSF+Plerixafor-mobilized HSCs transduced with the GLOBE vector, featuring a different design and incorporating only the HS2 and HS3 elements of the β-globin LCR [20]. The trial introduced two innovations: the conditioning regimen, which consisted of Treosulfan and Thiotepa instead of Busulfan, and an intra-osseous delivery of the gene-corrected cell preparation. How these innovations contributed to the outcome of the trial is not well understood. As of May 2018, nine patients have been treated with significant reduction in transfusion requirement in adults and transfusion independence in 4 out of 5 pediatric patients [21].

The results obtained in β-thalassemia encouraged attempts to extend gene therapy also to SCD patients. A first subject was treated in France with the BB305 LV vector: he received full myeloablativive chemotherapy with Busulfan and was transplanted with 5.6 × 10^6 of BM-derived CD34+ cells/kg transduced at an average VCN of 1.1. The patient rapidly achieved a level of therapeutic βT87Q globin around 50% and transfusion independence, with a clinical picture comparable to that of an SCD carrier [9]. Integration site analysis showed polyclonal hematopoietic reconstitution and no clonal abnormality. Unfortunately, a subsequent, multicenter clinical trial carried out in the USA with the same vector failed to achieve a level of correction comparable to that observed in the first patient. Patients received a median dose of 2 × 10^6 of BM-derived CD34+ cells/kg transduced at a median VCN of 0.6. A follow-up of 8 to 17 months showed a VCN in peripheral blood of <0.12, HbAT87Q levels of <2 g/dl and an average HbAT87Q/HbS (sickle hemoglobin) ratio of <15% [22]. These results pointed to the difficulty in obtaining adequate doses and robust engraftment of transduced HSCs in SCD patients, particularly adult subjects.

Despite these promising results, several issues remain unresolved and limit the efficacy and a more widespread use of gene replacement therapy for β-hemoglobinopathies, and particularly for the most severe form of β-thalassemia (β0/β0 genotype) and for SCD. Among those, procurement and quality of the HSCs, the toxicity associated to BM conditioning and the complexity of the cell manufacturing process, which impact on the efficacy, tolerability, cost and widespread availability of gene therapy.

**THE HSC MOBILIZATION PROCESS**

In β-hemoglobinopathies, anemia is due to the association of two pathological processes: the peripheral hemolysis, which can be intra and extra vascular, and the BM impaired ability to produce terminally differentiated erythrocytes – a defect referred to as dyserythropoiesis or ineffective erythropoiesis [23–25]. The first consequence of dyserythropoiesis is the accumulation of erythroid progenitors: the BM of patients suffering from β-thalassemia contains five to six times more erythroid precursors than normal. The altered BM composition explains the initial failure to provide an appropriate HSC harvest for gene therapy. In SCD, the best evidence of dyserythropoiesis comes from patients treated with
allogeneic HSC transplantation, who displayed an early selection pressure in favor of donor erythroid precursors and a reduced survival of the host precursors [26,27].

Replacing BM harvest with HSC mobilization has been successfully achieved in β-thalassemia patients by the use of G-CSF or a combination of G-CSF+Plerixafor. Attempts to use G-CSF in SCD patients caused, however, vaso-occlusive crises and at least one fatality, thus G-CSF is no longer used. Recently, a clinical trial has showed that Plerixafor alone can be safely used to mobilize HSCs in young SCD patients, opening the road to mobilization as an alternative to BM harvest also in SCD [28]. Some limitations exist, however, also in the use of Plerixafor: patients should be hyper-exchanged before the collection in order to reduce the HbS level to <30%, and tightly monitored in terms of hydration, electrolyte metabolism and kinetics of CD34+ cell egress. Moreover, it is still not known if Plerixafor can also be safely administered to adult patients, particularly those suffering from severe vasculopathies, who may be more prone to vaso-occlusive crises with life-threatening consequences.

The superior quality of HSCs mobilized by Plerixafor versus those mobilized by G-CSF has been shown in the context of different inherited diseases [29]. Plerixafor-mobilized HSCs contain a highest frequency of NSG mouse-repopulating cells in stringent limiting-dilution assays and have a higher homing capacity with respect to cells mobilized by G-CSF only or a combination of G-CSF+Plerixafor [29]. Interestingly, Plerixafor-mobilized cells showed a more prevalent HSC signature compared to other conditions [28,29], confirming the superiority of this mobilization modality.

The kinetics of CD34+ cell mobilization after administration of G-CSF, Plerixafor or their combination depends on the underlying disease and on the presence or absence of inflammation, a factor influencing the extent of the immature CD34+ compartment. In the presence of inflammation, the level of CD34+ cells in peripheral blood should be tightly monitored to optimize the cell harvest, adding an additional element of complexity to the procedure that requires specialized centers to be carried out safely and efficiently. Additional research is clearly needed to identify new mobilization agents or combination thereof, to mobilize HSCs more rapidly and efficiently, and ideally independently form patient’s genetic and phenotypic background.

**BONE MARROW CONDITIONING**

Autologous transplantation of genetically modified HSCs is associated to dramatically reduced risks of immune-mediated complications as compared to allogeneic transplantation. Nevertheless, the procedure still requires BM conditioning to ‘make space’ to, and induce a selective pressure in favor of, the incoming HSCs. While low-dose conditioning is sufficient to create a mixed chimerism when gene-corrected cells have a natural selective advantage (e.g., in T-cell immunodeficiencies), diseases such as β hemoglobinopathies require fully myeloablative doses of conditioning drugs to achieve therapeutic levels of engraftment. A single-drug...
regimen based on the use of Busulfan with tight monitoring of the pharmacokinetics to maintain the dose at myeloablative levels achieves sufficient engraftment of genetically corrected cells while significantly limiting T-cell depletion, and therefore immune suppression. None of the patients undergoing gene therapy for β-thalassemia or SCD in the current clinical trials has experienced severe post-transplant infections thanks to the continuous persistence of a protective T-cell immunity.

Despite the advantages of Busulfan-only regimens with respect to fully myelo- and immune-ablative protocols used in allogeneic HSC transplantation, the drug still induces systemic cell toxicity, causing transitory epithelial and mucosal inflammation and gastro-intestinal symptoms and, importantly, sexual hormone insufficiency. Patients require germinal tissue cryopreservation before starting the procedure, and a long-term hormone replacement therapy. The long-term consequences of Busulfan treatment are an important factor in a risk–benefit evaluation and justify more fundamental research in the identification of non-chemical myeloablative agents that may allow efficient HSC grafting while sparing fertility and reducing or eliminating systemic toxicity. Conditioning based on the use of specific monoclonal antibodies, such as anti-CD117 [30], is able to deplete BM HSCs without extra medullary toxicity and is currently tested in a Phase 1 clinical trial of pheno- or haplo-identical HSC transplantation for SCID patients (NCT02963064). Other immunotherapies, using a saporin-conjugated anti-CD45 antibody [31], or a combination of CD47 blockade and anti-c-kit antibody (ACK2) [32], have demonstrated their efficacy and safety in murine models. These potentially less toxic conditioning regimens could simplify HSC gene therapy and further expand its range of applicability.

CELL MANUFACTURING & THE REGULATORY FRAMEWORK

The biggest challenge in the development of gene therapy for β-thalassemia and SCD is the development of acceptable modalities for its delivery to patients, which should include widespread availability and reasonable prices. The manufacturing of a gene-modified HSC preparation is a complex process: it starts with harvesting peripheral mobilized mononuclear cells by leukapheresis, followed by immunoselection of an HSC-rich CD34+ cell fraction, activation in culture in the presence of a cocktail of cytokines, transduction with a LV under appropriate conditions and finally cryopreservation. The transduced cell batch is then quality checked for a number of parameters, most importantly the transduction efficiency, and eventually released for clinical use. Each one of these steps is subjected to substantial individual donor variability, and has a risk of failure that can result in a failed production run.

Cell manufacturing requires relatively long ex vivo culture with exposure to cytokines and high vector doses, which impacts on HSC biological properties by increasing apoptosis and decreasing engraftment capacity [33,34] to various extent, depending on the disease context [35]. Additives such as
cyclosporine A, rapamycin, SR1, UM171 or PGE2 improved transduction and properties of HSCs in pre-clinical studies [36–40]. To note, PGE2 is currently tested in gene therapy trials for β-thalassemia (HGB207, HGB212).

Legislation introduced a decade ago in Europe and the US demands that gene-corrected CD34+ cell preparations are produced under the same good manufacturing practices (GMP) developed for chemical drugs, in pharmaceutical establishments built and operated with industry-like standards and licensed by governmental agencies. However, this complex combination of viruses and patient's cells is essentially a genetically engineered autologous transplant, a personalized medicine by definition more than a pharmaceutical 'product'. The reason for the GMP requirement is only the genetic modification, since non genetically modified CD34+ cell preparations are considered 'minimally manipulated' and prepared in hospitals under non-GMP standards. This regulatory framework is a formidable challenge for the commercial development of gene therapy for blood diseases, as it restricts the production of gene-corrected cells to manufacturing facilities that are extremely expensive to build and operate and creates logistic constraints that severely limit the applicability of the technology. The European 'hospital exemption' clause, or its equivalents elsewhere in the world, allows hospitals to produce limited quantities of genetically modified cell products under the authority of a medical practitioner for individual patient's use, but cannot be the basis for a commercial use of the products and is essentially used only in compassionate contexts. In practice, unless an industry decides to embark in the full production cycle from the vector to the gene-corrected cells to the logistics of delivering the product to the end users, there is no chance for this form of therapy to be marketed and made available to patients as a standard form of therapy. Last but not least, rare diseases with a limited patient base are not an attractive target for a commercial entity and are unlikely to be developed and marketed unless with restricted delivery modalities as in the case of Strimvelis®, which is manufactured only in one establishment and is available only in one country. The complexity of having cells shipped back and forth to the manufacturing center or patients traveling from remote for long periods of time remains a significant obstacle to widespread application of a therapy delivered with such restricted modalities.

The existing regulatory constraints are practically limiting the chances that ex vivo gene therapy with genetically modified HSCs becomes an accepted standard of care, even in the presence of compelling evidence of safety and efficacy. The manufacturing of gene-modified HSCs did not significantly evolve in the last two decades and is still based on cumbersome, labor-intensive, expensive and relatively small-scale processes. The academia that developed the ground-breaking transfection-based production systems for retroviral vectors and the ex vivo HSC manipulation technology is ill-positioned to evolve those basic concepts into robust, large-scale industrial processes that may improve quality and reduce manufacturing costs.
An emerging contract manufacturing industry is currently limiting itself to adapting processes transferred by clients, with little or no innovation and a still very limited production capacity. The biotechnology and pharma industry are the only players that can afford the significant investment required to develop commercial manufacturing technology, but again, they are unlikely to engage in the exercise in the absence of a potential return on the investment, which may materialize only for very few diseases, such as, for example, SCD. Notably, the next-best technology to genetically modify HSCs, i.e., gene editing, will soon face the same kind of problems, and although it will reduce the complexity of the current gene addition technology by eliminating the viral vector component, it will still require GMP cell manufacturing to be implemented, with likely similar issues of costs and logistics.

A possible solution to this conundrum is a combination of technological and regulatory evolution. Automating HSC purification and transduction in a closed, controlled environment that does not require open-air manufacturing steps is certainly possible, and prototype machine and processes have been developed to prove this concept [41–45]. Most likely, the CAR-T cell manufacturing technology will drive this automation process, and eventually benefit also the HSC field. When available, a closed-circuit device could operate in a decentralized fashion in accredited hospitals and transplantation centers, reducing to a minimum the complexity of centralized cell manufacturing. Manufacturing of all non-patient-specific components, such as the viral vectors and all ancillary reagents such as culture media, cytokines and transduction enhancers, would remain centralized and subjected to the standard industrial practices of quality control and batch release. An automated, patient-specific, hospital-based cell manufacturing should be seen as a reasonable alternative to centralized GMP manufacturing, and evaluated and approved as such by regulatory agencies worldwide.

The advantages of a decentralized manufacturing model are multiple and important. First of all, the biotech/pharma industry would be responsible for the production of a genetic vector only, not of individual patient’s cells, and could potentially ‘license’ cell manufacturing to centers meeting quality standards through an accreditation process. Second, it would allow a more rapid evolution of vector design and manufacturing for any given disease, which would become uncoupled from cell manufacturing and no longer bound into a single ‘product’. Likewise, innovation in HSC culture, expansion and genetic modification will be free to evolve independently from a specific vector and be pursued more efficiently and by potentially different actors. Last but not least, any clinical center in any country could have a chance to deliver gene therapy to patients without asking them to embark in expensive and often traumatic travel to foreign countries, and do so independently from the logistic constraints and cost of centralized cell manufacturing. Clinical centers could become the actual ‘manufacturers’ of the final product, as they currently are for any other form of transplantation medicine, and
possibly additional actors of innovation and evolution.

CONCLUDING REMARKS

Gene therapy for hemoglobinopathies is showing its safety and potential efficacy in early clinical studies. However, source, quality and dose of repopulating stem cells, suboptimal transduction efficiency and gene expression levels, and toxicity and efficacy of current conditioning regimens remain significant hurdles limiting generalized application, particularly in the most severe thalassemia patients and in SCD. In addition, the complexity and costs associated to vector and cell manufacturing and an ill-adapted regulatory environment are limiting the applicability and commercial future of this therapeutic approach, particularly in less developed countries. Despite the encouraging results and the current enthusiasm by both investigators and industrial sponsors, these limiting factors need be systematically addressed for gene therapy of hemoglobinopathies to become a clinical reality. The emerging gene editing technology may overcome at least some of these limitations and provide additional therapeutic alternatives, although its safety and efficacy are yet to be tested in the clinical reality.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

The authors have no relevant financial involvement with an organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock options or ownership, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

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