Gene Therapy for Hemoglobinopathies: Tremendous Successes and Remaining Caveats

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β-globinopathies (sickle-cell anemia and β-thalassemia) are the most common monogenic disorders worldwide that are potentially amenable to gene therapy. Proof-of-concept studies done 15 years ago, which showed correction of murine models of hemoglobinopathies, have gone through the rigors of preclinical safety and efficacy testing, and are now showing clinical promise both in β-thalassemia and sickle-cell anemia. Lentivirus-mediated gene replacement therapy with erythroid-specific expression of the globin gene appears safe thus far. However, both cures and modest successes were reported at two recent meetings (the Tenth Cooley's Anemia Symposium, 18–22 October 2015, and the 57th American Society of Hematology (ASH) Meeting, 5–8 December 2015) for both thalassemia and sickle-cell anemia (SCA), demonstrating that some caveats remain to be addressed before gene replacement therapy can potentially cure all patients with β-globinopathies.

Hemoglobinopathies are a large, heterogeneous group of inherited disorders of hemoglobin synthesis that comprise thalassemia syndromes and structural hemoglobin variants. Thalassemia syndromes result from diminished or absence of α- or β-globin proteins, leading to α-thalassemia or β-thalassemia, respectively. Structural hemoglobin variants result from alteration of the globin protein, leading to its abnormal function. One such structural variant leads to SCA, where a point mutation in the β-globin gene leads to its polymerization upon deoxygenation, turning the normally disc-shaped red blood cells into sickle shapes that occlude microvasculature. Disorders of β-globin synthesis (β-globinopathies) cause immense morbidity and mortality worldwide, with the highest density of the disease present in the tropical regions of the world.[1,2] According to a World Health Organization report, approximately 5% of the world's population carries trait genes for hemoglobin disorders, mainly SCA and thalassemia.[3] Approximately 100,000 Americans have SCA, whereas in sub-Saharan Africa more than 300,000 affected infants are born yearly.[4,5]

Hematopoietic stem cell (HSC) transplant from a matched-sibling donor can cure both SCA and β-thalassemia effectively. However, availability of matched donors is limited to only a fraction (10–20%) of patients and is accompanied by potential immunological side effects (graft-vs.-host disease or graft rejection). Hence, the vast majority of β-thalassemia patients that produce greatly diminished β-globin (β^+-thalassemia of $\beta^E\beta^0$-thalassemia), or no β-globin (β^0-thalassemia) remain on lifelong transfusions every 3–4 weeks and iron chelation therapy. Those with SCA suffer from severe painful vaso-occlusive events and are often placed on daily hydroxyurea therapy to reactivate fetal (γ)-globin production, in that γ-globin prevents polymerization of sickle hemoglobin and ameliorates SCA phenotype. Hence, these monogenic defects in β-globin are prime targets for gene therapy, where replacement of a normal β-, a modified β-, or a γ-globin gene can restore normal hemoglobin levels/function.

After nearly 15 years of failed attempts at replacing the globin gene and its regulatory elements using γ-retrovirus vectors, lentivirus vectors were shown to correct the mouse thalassemia and sickle-cell disease phenotype.[6,7] Over the years, preclinical data from several laboratories using murine models[6–11] and experimental human models (*in vitro* models and xenograft models)[12–14] of these diseases have demonstrated both the efficacy and safety of several different lentiviral vectors driving expression of β-like globin genes or their modified versions that confer on them anti-sickling properties.[6–18] All of these lentivirus vectors are driven by the β-globin gene promoter and carry the locus control region elements to express in an erythroid-specific manner.

The results of an initial clinical trial for β-thalassemia and sickle-cell disease using a lentivirus termed LentiGlobin (also known as BB305) were presented at the ASH meeting in December 2015. The first subject suffering from transfusion-dependent hemoglobin E-β-thalassemia (β^E/β^0-thalassemia) was infused with autologous $CD34^+$ cells that had been transfected with the lentivector expressing β^{T87Q}-globin in June 2007 following myeloablative conditioning. The patient became transfusion independent a year later and has remained largely transfusion independent for 7 years. The subject has maintained a hemoglobin of 9–10 g/dl, a level that is consistent with mild anemia but sufficient to confer transfusion independence. The therapeutic benefit was initially attributed to expansion of a clone with increased expression of the HMGA2 gene due to vector insertion. This HMGA2 clonal dominance was benign, however, and subsided, while new dominant clones emerged, consistent with the dynamic fluctuation of HSC clones.[18] These results are consistent with the proven safety record of lentivirus vectors used to treat patients with adrenoleukodystrophy,[19] metachromatic leukodystrophy,[20] and Wiskott-Aldrich syndrome.[21]

The trial was extended to include 10 patients with transfusion-dependent $\beta^E\beta^0$, 5 subjects with $\beta^0\beta^0$, and 3 with β^+-thalassemia, and 4 with SCA.[22–25] All patients received myeloablative conditioning with busulfan. Mobilized peripheral blood–derived $CD34^+$ cells were the HSC source in the β-thalassemia patients, whereas bone marrow–derived $CD34^+$ cells were the HSC source for those with SCA. Subjects with β^E/β^0-thalassemia and severe β^+-thalassemia experienced a 4.9-g/dl median rise in hemoglobin and became transfusion independent within a year. By contrast, patients with β^0/β^0-thalassemia

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all experienced reductions in transfusion requirements by an average of 30–50%.

Overall, the most recent analysis suggests that LentiGlobin-transduced HSCs support the production of an average of 5 g/dl hemoglobin in both non-β^0/β^0 and β^0/β^0-thalassemia genotypes with one or two vector copies per cell. This rise in hemoglobin was adequate for the less severe β-thalassemia major phenotypes ($\beta^E\beta^0$ or β^+ compound heterozygotes), but the level of expression was insufficient to completely correct $\beta^0\beta^0$-thalassemia (when the endogenous β-globin production is absent), where significant reduction in transfusion requirements nevertheless occurs. Increasing the vector copies per cell to further boost hemoglobin production might increase the risk of insertional mutagenesis; and increasing chemotherapy conditioning (busulfan exposure) to completely ablate the endogenous HSC pool, to allow engraftment only of gene-marked cells, would greatly increase the number of days to hematopoietic recovery, and increase morbidity and possibly risk mortality. Certainly this approach will not be readily transportable to low-resource countries. Hence, improved expression from the vector may achieve success in these severely affected patients.

LentiGlobin was also used to treat four patients with SCA, again in the context of myeloablative conditioning with busulfan, with divergent results. The first patient treated in France exhibited substantial clinical benefit over the first year post treatment and remains completely free of disease-related adverse events. Indeed, nearly half the hemoglobin in this patient is β^{Q87T}, with remarkable improvement in the phenotype. However, three subsequent patients treated in the United States did not show the same degree of success, possibly as a result of a lower number of HSCs infused, and lower engraftment of gene-modified cells. The β^{Q87T}-globin percentage achieved was low (2–10%) but is gradually increasing. These patients are all within 9 months of transplant, and there may yet be a positive selection of gene-modified cells over time. Important differences exist between the one complete success from France and the three others with modest increases in β^{Q87T}-hemoglobin. The French patient received a high dose of 5.6 million $CD34^+$ cells/kg that exhibited vector copy numbers of ~1.0 that were sustained *in vivo*, suggesting that only *ex vivo*–manipulated cells contributed to the graft. Furthermore, this patient exhibited a high degree of myeloablation with a markedly prolonged time before peripheral blood count recovery. The results suggest the importance of a high initial gene-modified HSC dose. Attempts at further increasing vector copy numbers per cell are predicted to increase the risk of longer term side effects such as dysregulated hematopoiesis due to insertional mutagenesis. Alternatively, more potent lentiviral vectors should be explored. Furthermore, safe mobilization of stem and progenitor cells is needed, especially in this disease, where frequent bone and bone marrow crises and avascular necrosis can greatly reduce the number of $CD34^+$ cells available from bone marrow harvests. Indeed, multiple bone marrow harvests were performed in the SCA adult patients in the United States to obtain sufficient HSCs for gene modification and transplant. Memorial Sloan Kettering has begun a phase I trial of plerixafor to mobilize hematopoietic stem and progenitor cells in patients with SCA (NCT02193191), results of which are eagerly awaited.

Four related vectors that have exhibited preclinical efficacy have also moved forward into clinical evaluation, and trials have opened in the United States and in Italy.

1. NCT01639690 (a normal β-globin–carrying vector for the treatment of β-thalassemia[6]). In this trial, chemotherapy conditioning (given to destroy the host hematopoietic cells) busulfan dose was reduced to nearly half that used for complete myeloablation. The rationale behind this nonmyeloablative conditioning is that partial chimerism of 11–25% normal HSCs has been shown to cure thalassemia[26,27] and SCA[28,29]; and a reduction in chemotherapy intensity will reduce the short- and long-term morbidity while allowing sufficient autologous gene-modified HSCs to engraft. Another difference in this trial was that the lentiviral gene transfer was performed over 4 days. Lentiviruses transduce quiescent HSCs, and hence a two-day gene transfer process is typically used for these vectors, whereas γ-retroviral vectors typically require a four- to five-day process to enforce HSC division *in vitro* to allow transduction. Three patients with β-thalassemia major have been treated with this vector with reduced-intensity conditioning with busulfan, following a four-day *ex vivo* gene transfer procedure. The engraftment of gene-modified cells was modest, despite administration of a large number of $CD34^+$ cells, with 2–8% of gene-modified red blood cells and some reduction in transfusion requirement in one patient. The protocol has now been amended for higher intensity chemotherapy conditioning and a shorter gene transfer procedure.[30]

2. NCT02453477 (vector encoding normal β-globin gene[31] for the treatment of β-thalassemia using full myeloablative conditioning, using a different chemotherapy regimen (composed of treosulfan and thiotepa)). One β-thalassemia major patient treated with the latter vector was transfusion-independent by 3 months.[32]

3. NCT02247843 (vector encoding a β-globin gene with three anti-sickling mutations[14,33] for the treatment of SCA using full myeloablative conditioning with busulfan).

4. NCT02186418 (a γ-globin lentivirus vector for the treatment of SCA[11] using reduced-intensity conditioning with another chemotherapy agent, melphalan).

The results of the latter two trials, now open, are eagerly awaited. All of these lentiviral vectors utilize a modified locus control region and β-promoter to drive expression of various types of globin genes.

In summary, gene replacement therapy is beginning to show clinical efficacy in those hemoglobinopathies that can be readily corrected with a 3- to 5-g/dl increase in hemoglobin. However, success in β^0-thalassemia or in SCA has only been achieved at the cost of profound myeloablative conditioning and infusion of large doses of gene-modified HSCs. Achieving higher vector copies per cell to increase hemoglobin production may not be clinically feasible and has the potential for insertional mutagenesis, while achieving higher HSC dose in adult patients with SCA may also not be feasible, until strategies that maintain or expand genetically manipulated HSCs *in vitro* can be applied, or ways to safely mobilize larger numbers of HSCs into circulation are developed. Improvements in the vector design to increase vector potency can improve the therapeutic efficacy. Newer gene editing efforts could help circumvent some of these issues, although they are still at early stages and have their own limitations. In the β-globinopathies, gene-corrected HSCs do not have a survival advantage, and therefore, a high degree of chimerism

for gene-modified HSCs is necessary. Furthermore, a high level of hemoglobin production per vector copy is also necessary. As such, the HSC dose, the type of chemotherapy conditioning, its intensity, and vector potency are all critical to success. There is certainly room for improvement, but it is clear that HIV-1-based vectors are becoming vehicles of successful therapeutics for hemoglobinopathies.

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