The Libyan International Medical University

Faculty of Basic Medical Science

Gene Therapy: Treatment of Sickle Cell Disease

Yousra Swedik

Supervised by: Dr. Majdi Sasi

Assisted by: Dr. Nada Elshelmany

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Abstract

Sickle cell disease is a disorder that results from a homozygous missense mutation in the β-globin gene that causes polymerization of hemoglobin S. The use of gene therapy in patients with this disorder is complicated. This is due to complex cellular abnormalities and challenges that may hurdle achieving effective and persistent inhibition of polymerization of hemoglobin S. This report describes a trial where a patient was treated with lentiviral vector–mediated addition of an anti-sickling β-globin gene into autologous hematopoietic stem cells. Adverse events were consistent with busulfan conditioning, but none were observed with the lentiviral vector. Fifteen months after treatment, the level of therapeutic anti-sickling β-globin remained high (approximately 50% of β-like–globin chains) without recurrence of sickle crises and with correction of the biologic hallmarks of the disease.

Introduction

'Gene therapy is "the use of genes as medicine". It involves the transfer of a working gene copy into specific cells of an individual in order to repair a faulty gene copy [1].' Since the 1980s, it has been emerging as a promising new technology which aims to treat and prevent many diseases and disorders, and in particular, those which are hereditary. In previous years, its potential and success in treating genetic disorders has rendered it one of the most prevalent and encouraging approaches in the field of genetics [2-5].

'If a mutated gene causes a necessary protein to be faulty or missing, gene therapy may be able to introduce a normal copy of the gene to restore the function of the protein [6].' In the treatment of diseases, gene therapy usually involves the use of viral vectors that act as carriers. Primarily, a corrected gene is inserted into a viral vector. Typically, that viral vector is genetically modified so that it infects the targeted cells to deliver the new gene, without causing disease to the cells and tissues inside the body. Once a faulty gene is identified, the viral vector is used to deliver a duplication of the correctly functioning gene to the targeted cells [6]. When the gene reaches the cells, transcription takes place in which specialized proteins make a copy of the DNA sequence in the gene in the form of messenger RNA. mRNA then transfers this genetic sequence outside the nucleus. Ultimately, translation takes place, and this is the phase in which the cell machinery reads the genetic sequence or instructions and synthesizes the desired proteins [7].

Typically, genetic diseases and disorders which are caused by a mutation or defect in a single gene are the most suitable candidates for this type of treatment [2, 8]. An example of such a disease is sickle cell disease [9]. Sickle cell disease is an autosomal recessive disease which alters the shape of red blood cells in the body so that they are no longer able to carry out their function. It affects millions of people worldwide and is among the most prevalent inherited monogenic disorders. Approximately 90,000 people in the United States have sickle cell disease, and worldwide more than 275,000 infants are born with the disease annually [9-10].

Although the treatment of sickle cell disease is still elusive, there have previously been clinical trials which have provided substantial proof that gene therapy could be a

successful approach in treating this disease. One clinical trial in particular, held by a biotechnology company called Bluebird Bio, has successfully treated a 13-year-old patient with sickle cell disease, and is the first to do so [11-13].

The aim of this study was to determine the suitability and potential of gene therapy in the treatment of sickle cell disease.

Materials & Methods

In this clinical trial, scientists used a lentiviral vector. The LentiGlobin BB305 vector is a self-inactivating lentiviral vector that encodes the human HBB variant $\beta^{A\text{-}T87Q}$. In addition to inhibiting HbS polymerization, the T87Q substitution allows for the β-globin chain of adult hemoglobin $(HbA)^{T87Q}$ to be differentially quantified by means of reverse-phase high-performance liquid chromatography [12]. This vector contained small portions of the Human Immunodeficiency Virus (HIV), which was particularly used because of its effectuality at delivering the new gene into cells. The vector was modified to prevent the patient from being infected by HIV [14].

A bone marrow harvest procedure was then carried out to provide the scientists with the patient's blood stem cells for gene transfer. This procedure was preceded by exchange transfusion, and bone marrow was obtained without clinical sequelae. The only grade 3 adverse event reported during this procedure was anemia. The blood stem cells were then separated from the liquid bone marrow in a laboratory. Subsequently, bone marrow-enriched CD34+ cells were transduced with the LentiGlobin BB305 vector. The mean vector copy numbers of transduced cells were 1.0 and 1.2 copies per cell [12]. This was done to introduce a copy of the correctly functioning hemoglobin gene into the DNA of the blood stem cells. This type of procedure is known as ex-vivo gene therapy [11, 13-14].

The scientists then used intravenous busulfan, a chemotherapy agent, to kill the blood stem cells in the body, thereby making space for the new, modified blood stem cells [12, 14]. After a 2-day washout period, the transduced CD34+ cells were injected and re-

introduced into the body. Red-cell transfusions were to be continued after transplantation until a large proportion of HbA^{T87Q} (25 to 30% of total hemoglobin) was detected [12].

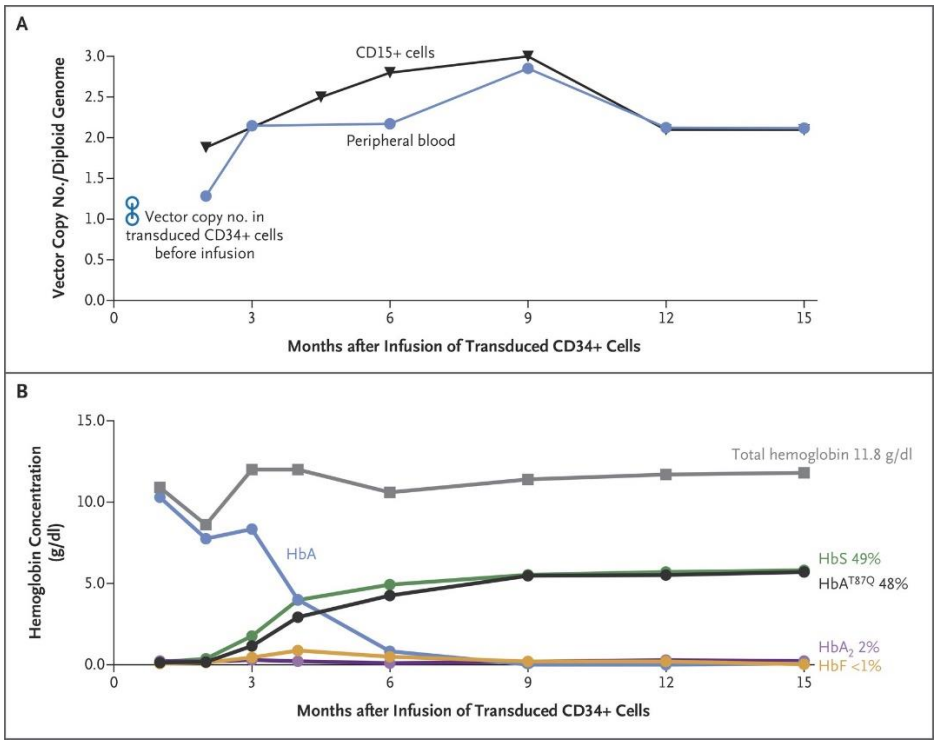
'The patient was followed for engraftment; toxic effects; vector copy number in total nucleated blood cells and in different lineages; and quantification of HbA^{T87Q} and HbS by means of high-performance liquid chromatography. Red-cell analyses were performed at month 12 [12].'

Results

Neutrophil engraftment was achieved on day 38 after transplantation, and platelet engraftment was achieved on day 91 after transplantation. Figure 1A shows the trajectory of vector copy numbers and Figure 1B shows production of HbA^{T87Q}. Gene marking increased progressively in whole blood, CD15 cells, B cells, and monocytes, stabilizing 3 months after transplantation. Increases in levels of vector-bearing T cells were more gradual [12].

Figure 1 shows production of HbA^{T87Q}. HbA^{T87Q} levels increased steadily and red-cell transfusions were discontinued, with the last transfusion on day 88. Levels of HbA^{T87Q} reached 5.5 g per deciliter (46%) at month 9 and continued to increase to 5.7 g per deciliter (48%) at month 15, with a reciprocal decrease in HbS levels to 5.5 g per deciliter (46%) at month 9 and 5.8 g per deciliter (49%) at month 15. Total hemoglobin levels were stable between 10.6 and 12.0 g per deciliter after post-transplantation month 6 [12].

Figure 1: Engraftment with Transduced Cells and Therapeutic Gene Expression



- 'Panel A shows vector copy number values in blood nucleated cells and the short-lived CD15+ (neutrophils) fraction thereof over 15 months after infusion of transduced CD34+ cells. Initial values in transduced cells before the infusion are shown [12]'.
- 'Panel B shows total hemoglobin levels and calculated levels of each hemoglobin fraction based on high-performance liquid chromatography measurements of globin chains. The percent contribution of hemoglobin fractions at month 15 is also indicated. The hemoglobin A (HbA) levels are derived from the regular red-cell transfusions received by the patient before gene therapy and briefly thereafter (last being on day 88). HbA2 is an alternative adult hemoglobin that is not derived from transfused blood. HbF denotes fetal hemoglobin, and HbS sickle hemoglobin [12]'.

Safety

'The patient had expected side effects from busulfan conditioning. Grade 3 and 4 events included grade 4 neutropenia, grade 3 anemia, grade 3 thrombocytopenia, and grade 3 infection with Staphylococcus epidermidis (with positive results on blood culture), all of which resolved with standard measures [12]'. In contrast to this, there were no observed adverse events related to the LentiGlobin BB305–transduced stem cells [12].

Clinical and Biologic Measures

The patient was discharged on day 50, and more than 15 months after transplantation, no sickle cell disease–related clinical events or hospitalization had occurred. All medications were discontinued, including pain medication. Following this, the patient reported full participation in normal academic and physical activities. Changes in sickle cell disease–related biologic measures are displayed in Table 1. Complete blood counts were stable, reticulocyte counts decreased substantially, and circulating erythroblasts were not detected. Laboratory values, including urinary microalbumin levels, indicated normal renal and liver functions. Plasma levels of total bilirubin and lactate dehydrogenase normalized. Soluble transferrin receptor levels improved and were 3.4 times as high as normal values at screening and 1.5 times as high at months 12 and 15, thus indicating progressive normalization of erythropoiesis [12].

Table 1: Laboratory Values before Gene Therapy (at Screening) and at 3-Month Intervals after Infusion of Transduced CD34+ Cells

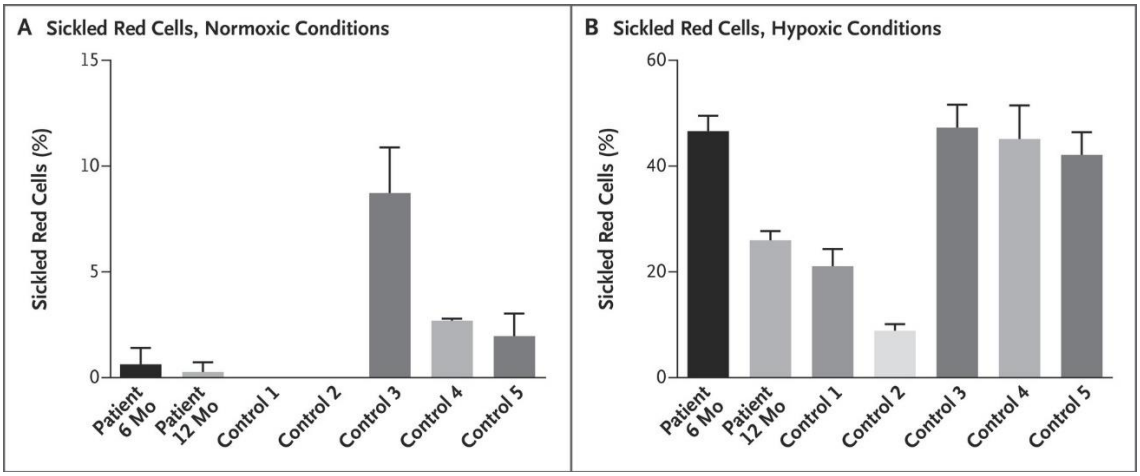
Table 1. Key Laboratory Values before Gene Therapy (at Screening) and at 3-Month Intervals after Infusion of Transduced CD34+ Cells.

Measure	Normal Range	Screening*	Month 3*	Month 6	Month 9	Month 12	Month 15
Total hemoglobin (g/dl)	13.0–18.0	10.1	12.0	10.6	11.4	11.7	11.8
Red-cell count (per mm^3)	4,500,000–6,200,000	3,700,000	3,900,000	3,700,000	4,000,000	4,200,000	4,300,000
Reticulocyte count (per mm^3)	20,000–80,000*	238,000	259,000	132,000	131,000	143,000	141,000
Mean corpuscular hemoglobin (pg/red cell)	25–30	28	31	29	28	28	28
Mean corpuscular hemoglobin concentration (g/dl)	31–34	35	34	35	36	35	35
Platelet count (per mm^3)	150,000–450,000	356,000	52,000	122,000	157,000	168,000	201,000
Neutrophil count (per mm^3)	1500–7000	4200	2400	3100	2500	3000	2200
Total bilirubin (μmol/liter)	0–17	50	15	20	14	12	12
Lactate dehydrogenase (U/liter)	125–243	626	285	254	226	274	212
C-reactive protein (ng/ml)	<500	191	814	129	ND	135	158
Ferritin (μg/liter)	22–275	265	869	1095	ND	520	363
Transferrin (g/liter)	1.9–3.2	1.4	1.6	1.7	ND	1.5	1.7
Transferrin saturation (%)	16–35	72	ND	ND	35	56	40
Serum transferrin receptor (mg/liter)	0.8–1.7	5.7	3.0	2.3	ND	2.6	2.5
Iron (μmol/liter)	12–30	26	18	14	ND	20	17
Hepcidin (ng/ml)	1–20	ND	ND	ND	ND	12.9	19.9
Alanine aminotransferase (U/liter)	5–45	22	76	125	116	53	41
Aspartate aminotransferase (U/liter)	5–40	53	57	71	50	49	35

* Exchange transfusion was performed before gene therapy was initiated, and supportive red-cell transfusions were completely discontinued 88 days after transplantation. ND denotes not done.

Being that the patient received a regular transfusion regimen for 4 years before this study and because of the exchange transfusion before transplantation, comparative studies before and after transplantation couldn't be conducted. Nonetheless, the proportions of sickled red cells in the patient's blood at months 6 and 12 were significantly lower than those in untreated patients with sickle cell disease (β^S/β^S). This is clearly demonstrated in Figure 2A. Moreover, at month 12, the sickling rate in hypoxic conditions was not significantly different from that of the patient's asymptomatic, heterozygous (β^A/β^S) mother, as indicated in Figure 2B [12].

Figure 2. Results of Sickle Cell Disease–Specific Red-Cell Assays



- 'Panel A shows rates of red-cell sickling under normoxic conditions (20% oxygen saturation) and Panel B shows rates of red-cell sickling under hypoxic conditions (10% oxygen saturation) in the patient at 6 months and 12 months after gene therapy and among control patients from whom red-cell samples were obtained: two patients with heterozygous A/S "sickle trait" (Controls 1 and 2; Control 1 is the patient's mother) and three patients with sickle cell disease (Controls 3, 4, and 5) [12]'.

Discussion

Hemoglobin is a protein molecule by which red blood cells transport oxygen from the lungs to the rest of the body's tissues. Red blood cells normally have a round shape and are very flexible. This is a very important characteristic because it allows red blood cells to travel through very narrow blood vessels [10-11]. The hemoglobin molecule is a tetramer with two alpha subunits and two beta subunits. In sickle cell disease, a gene found on chromosome 11, which codes for the beta component in hemoglobin, is mutated. This occurs when a valine replaces glutamate in position 6 of the beta subunit; the replacement is referred to as Glu6Val. This single amino acid substitution stems from a single base substitution (A→T) in the first exon of the human β^A-globin gene (HBB). Ultimately, this mutation alters the way in which the hemoglobin protein is formed (polymerization of hemoglobin S – atypical), thus leading to a deformity in the shape of red blood cells [10-12]. Instead of having a round shape, the red blood cells develop into a rigid and crescent or sickle-like shape. Patients generally have intense vaso-occlusive crises, leading to irreversible organ damage and poor quality of life [12-13].

This case report of a patient with sickle cell disease who underwent treatment by gene therapy with the use of lentiviral gene addition of an anti-sickling β-globin variant provides proof of concept for this approach. Once the transduced stem cells were engrafted, normal blood-cell counts were attained in all lineages. Additionally, increasing levels of vector-bearing nucleated cells in the blood over the first 3 months after transplantation and general vector copy number stability through month 15 suggest engraftment of transduced stem cells that were capable of long-term repopulation. No adverse events that were recorded by the investigators to be related to the BB305-transduced cells were observed, and the pattern of vector integration remained polyclonal without clonal dominance [12, 15].

The appearance of vector-bearing cells in the periphery corresponds to the time frame for engraftment of long-term progenitors and stem cells repopulating the space of nucleated cells. As compared to this, the slower pace for the increase of HbA^{T87Q} expression reflects the more gradual time course of replacement of transfused red cells from the pre-transplantation and peri-transplantation periods by newly matured, graft-derived red cells.

Further data on LentiGlobin treatment in sickle cell disease is currently being collected in HGB-206, a multicenter, phase 1/2 clinical study in the United States. Follow-up is more limited for these patients than for the patient in this study, but initial reports in seven patients have not included any new safety findings. Gene-transfer efficiency was lower than reported here, although therapeutic gene expression remained correlated with vector copy number values [12].

Outcomes in this clinical trial provide further supportive evidence to previously reported results of patients who underwent a similar ex vivo gene therapy procedure for β-thalassemia with the same BB305 vector. In addition to the patient with sickle cell disease described here, under this same clinical protocol, 4 patients with transfusion-dependent β-thalassemia have received LentiGlobin BB305. These participants had no clinically significant complications and no longer require regular transfusions. Moreover, these findings are also consistent with early results reported with 18 other patients with thalassemia who received LentiGlobin BB305 in clinical study HGB-204 [12].

Longer follow-up is required to confirm the durability of safety profile observed, and data from additional evaluations of gene therapy in a larger cohort of patients to confirm the promise of gene therapy are lacking. To sum up, this clinical trial shows substantial potential in helping to guide the design of future trials of gene therapy for sickle cell disease [12].

Conclusion

Although several of the initial obstacles to sickle cell disease gene therapy appear to have been overcome, it is prudent to recognize barriers that remain. Efficient transduction of hematopoietic stem cells with lentiviral vectors has become increasingly reliable, but the complicated components of many globin vectors present unique challenges for production of viruses capable of strong transduction. Safety and efficacy can only be established by careful clinical trials with extended patient follow-up. Gene engineering methods are advancing rapidly and should make possible the development of 2nd generation gene therapy approaches in upcoming years. It can be concluded that

following many years of preclinical laboratory investigation, gene therapy options are very propitious and now on the horizon for patients with sickle cell disease [12-13].

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