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Modified lentiviral globin gene therapy for pediatric β^0/β^0 transfusion-dependent β-thalassemia: A single-center, single-arm pilot trial

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SUMMARY

β^0/β^0 thalassemia is the most severe type of transfusion-dependent β-thalassemia (TDT) and is still a challenge facing lentiviral gene therapy. Here, we report the interim analysis of a single-center, single-arm pilot trial (NCT05015920) evaluating the safety and efficacy of a β-globin expression-optimized and insulator-engineered lentivirus-modified cell product (BD211) in β^0/β^0 TDT. Two female children were enrolled, infused with BD211, and followed up for an average of 25.5 months. Engraftment of genetically modified hematopoietic stem and progenitor cells was successful and sustained in both patients. No unexpected safety issues occurred during conditioning or after infusion. Both patients achieved transfusion independence for over 22 months. The treatment extended the lifespan of red blood cells by over 42 days. Single-cell DNA/RNA-sequencing analysis of the dynamic changes of gene-modified cells, transgene expression, and oncogene activation showed no notable adverse effects. Optimized lentiviral gene therapy may safely and effectively treat all β-thalassemia.

INTRODUCTION

Transfusion-dependent β-thalassemia (TDT) is among the most common monogenic diseases worldwide, particularly in the Eastern Mediterranean region, Southeast Asia, and Southern China, with approximately 60,000 patients diagnosed annually.[1,2] TDT is caused by mutations in the β-globin gene (*HBB*) with either reduced (β^+) or absent (β^0) production of functional β-globin.[1] Abnormal production of β-globin alters the balance between β-globin and α-globin chains and leads to an excess of unpaired α-globin chains that aggregate and form precipitates, resulting in damaged red cell membranes and intravascular hemolysis.[3,4] Traditionally, patients with TDT require lifelong red blood cell (RBC) transfusions and regular iron chelation. Although allogeneic hematopoietic stem-cell transplantation (HSCT) from a human leukocyte antigen (HLA)-matched donor can provide a cure for TDT, this treatment is limited by insufficient donors and the risks of transplantation-related death, graft failure, and graft-versus-host disease.[5,6]

Therefore, gene therapy (GT) for modifying autologous $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) has been developed to overcome the limitations of allogeneic transplantation. Upregulation of γ-globin via CRISPR to mimic the hereditary persistence of fetal hemoglobin (HbF) and thus reduce the severity of sickle-cell disease and thalassemia has recently been evaluated in clinical trials and has shown early evidence of efficacy and safety.[7,8] However, as is nature's way, γ-globin is gradually silenced after birth, and the long-term outcomes resulting from persistently high levels of γ-globin expression in adulthood, especially in pregnant women who may deliver growth-restricted or small for gestational age fetuses when the mother and the fetus have equivalent oxygen affinities, remain to be carefully evaluated.[9,10] Alternatively, autologous transplantation of healthy β-globin gene-added HSPCs, which directly mimic the globin status of healthy adults, has been reported to result in sustained and high β-globin levels and enable transfusion independence in most patients with a non-β^0/β^0 genotype thalassemia, without notable side effects in a long-term follow-up study.[11–13]

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However, for the most severe β^0/β^0 genotype thalassemia, lentivirus-mediated GT led to transfusion independence in only one-third of the patients in the Northstar trial (HGB204).[14] Although the ongoing Northstar-3 trial (HGB212), which uses the same lentiviral vector (LVV) as for the non-β^0/β^0 trial but with an optimized manufacturing process, may have obtained improved clinical outcomes in β^0/β^0 patients, the efficacy and safety report remain to be published.[15,16] The β^0/β^0 thalassemia is characterized by a complete loss of β-chain production due to deletion, initiation codon, nonsense, frameshift, or splicing mutations in the *HBB* gene, suggesting that further augmentation of β-globin expression via improved vector design or transduction is necessary to completely reverse the disease phenotype.[17]

To cure β^0/β^0 thalassemia by gene addition, in this study, we engineered an LVV to increase *HBB* expression (designated BDLG-04) and evaluated its safety and efficacy in two children with β^0/β^0 thalassemia. The BDLG-04 vector was also ligated with a short insulator in the 3′-long terminal repeat (LTR) to potentially minimize the risk of insertional mutagenesis. Both patients maintained an average of 2–4 copy numbers of the drug product (DP) per diploid genome from the first month until their last follow-up visits. Additional analysis with single-cell DNA sequencing of peripheral blood mononuclear cells (PBMCs) at subsequent visits showed that the transgene-inserted cells were in the range of 33%–65%. The insertion profile of the LVV in the patients revealed by tagmentation-assisted PCR (tag-PCR) analysis of PBMCs did not find dominant clones. In addition, single-cell RNA sequencing (scRNA-seq) and comprehensive analysis of the transcriptomes reconstituted from the infused HSPCs showed that lentiviral transduction did not lead to substantial transcriptional changes in the blood lineages. In summary, our study suggests that the optimized lentiviral globin GT product BD211 may serve as an effective therapy for patients with β^0/β^0 genotype TDT.

RESULTS

Optimizing LVV for β-globin expression *in vitro* and *in vivo*

To achieve efficient and stable transfer of the β-globin gene leading to therapeutic level of transgene expression in TDT patients, we set out to obtain an LVV with optimal HBB expression and potentially less susceptible to clonal dominance formation by comparing different designs of HBB cassettes with varying lengths of local control regions (LCR), HBB promoters, and introns (Figure S1A). A relatively long regulatory region is mandatory to drive efficient HBB expression. However, the overlong sequence may sacrifice the titer of LVV. Therefore, we also constructed an extremely short expression cassette containing the BCL11A enhancer and glycophorin A (GPA) promoter to ensure erythroid-specific expression of the HBB cDNA (Figure S1A). All candidate constructs were inserted with a short insulator derived from foamy viruses (FVs), which showed reduced genotoxicity potential in the U3 region of the LTR (Figures S1A–S1C).

We first compared BDLG-01 and BDLG-02, which differed in the length of LCR—2.6 and 3 kb, respectively. We found that the 3 kb-LCR composed of HS4-HS3-HS2 was more potent in driving HBB expression (Figure S1D). We then selected the 3 kb-LCR as an enhancer and compared varied designs (BDLG-02∼BDLG-05) for the relative HBB expression and found that BDLG-04 exhibited the highest HBB expression among all the constructs (Figures S1D and S1E). Therefore, we chose BDLG-04 for the subsequent study and confirmed the HBB expression at different multiplicity of infection (MOI) via reverse-transcriptase quantitative PCR (RT-qPCR) and western blot, respectively (Figures S1F and S1G). Under the condition of MOI 100 and lentiBoost for hematopoietic stem cell (HSC) transduction, the HBBT87Q mRNA transcribed from the LG04 vector could be over 40% of the endogenous *HBB* mRNA in the HSC-derived erythroid lineage (Figure S1F). Finally, we evaluated the efficacy of BDLG-04 in thalassemia mice by isolating and transducing Sca-1^+ cells and then transplanting the successfully transduced mouse HSCs back into the recipient mice (Figures S2A and S2B). We found that BDLG-04 significantly improved the body weight of the thalassemia mice (Figure S2C). Importantly, BDLG-04-modified HPSC reconstituted normal hematopoiesis in mice and substantially improved HBB expression close to the level of wild-type mice (Figures S2D–S2L). Based on the above study, we chose BDLG-04 for the clinical trial and designated the BDLG-04 modified HSC DP as BD211.

Clinical outcomes in the two patients after BD211 therapy

Patients diagnosed with β-thalassemia major were enrolled in the clinical trial according to the inclusion and exclusion criteria registered at ClinicalTrials.gov (identifier: NCT05015920) with the informed consent of their legal guardians. The study was approved by the Medical Ethics Committee of the 920th Hospital of Joint Logistics Support Force of People's Liberation Army of China in Kunming on April 15, 2021. In 2021–2022, two patients with β^0/β^0 genotype TDT were treated in this study: one in September 2021 and another in February 2022 (Table 1). The detailed description of the inclusion/exclusion criteria is given in the clinical protocol. A schematic diagram of this clinical trial as well as the insulator-carrying vector design is provided (Figures 1A, S1, and S2). The follow-up after transplantation was 28 and 23 months, respectively. Patient 1 (P1) was 11 years old and had the 17M/41–42M genotype (mutations in *HBB*: codon 17[A>T]/codon 41/42[-TTCT]) with an annual packed RBC (pRBC) transfusion history of approximately 36 units per year over 2 years prior to enrolment in this study. Patient 2 (P2) was 7 years old and also had the 17M/41–42M genotype, with an annual pRBC transfusion history of about 36 units per year over 2 years before enrolment in this study (see Table 1 for additional patient information). Neither patient had undergone splenectomy.

To evaluate the safety and efficacy of BD211, we performed autologous HSCT using BDLG-04-modified $CD34^+$ HSPCs from the mPBSCs of the two β^0/β^0 patients. Both patients received single-agent, pharmacokinetically adjusted, intravenous busulfan myeloablation for 4 consecutive days prior to a single intravenous infusion of modified autologous $CD34^+$ HSPCs (BD211). P1 received a low dose of 2.63×10^6 $CD34^+$ cells/kg, while P2 received a high dose of 7.92×10^6 $CD34^+$ cells/kg. Additional information regarding the production and infusion of the modified $CD34^+$ HSPCs is provided in Table 1.

We evaluated the percentage of genetically modified cells before and after transplantation by single-cell DNA sequencing. The HBB^{T87Q} -positive cells accounted for 59.48% and 65.16%