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Targeted CRISPR-Cas9 Non-Viral Epigenetic Reactivation of Fetal Hemoglobin: Long-Term Safety and Pharmacodynamics in Transfusion-Dependent Beta-Thalassemia

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ABSTRACT

Background: Transfusion-dependent beta-thalassemia (TDT) requires lifelong red blood cell transfusions and iron chelation. Non-viral electroporation of autologous CD34+ hematopoietic stem cells targeting the erythroid-specific BCL11A enhancer via Cas9 ribonucleoproteins demonstrated sustained transfusion independence in 92.8% of patients.

KEYWORDS: CRISPR-Cas9, Beta-Thalassemia, Fetal Hemoglobin, BCL11A Enhancer, Gene Editing Safety

1. INTRODUCTION & THEORETICAL CONTEXT

Transfusion-dependent beta-thalassemia (TDT) is an inherited monogenic hemoglobinopathy characterized by severe deficiency or complete absence of functional beta-globin chain synthesis. This molecular defect triggers unbalanced alpha-globin precipitation, ineffective erythropoiesis in bone marrow, chronic hemolytic anemia, systemic iron overload, and progressive end-organ damage requiring lifelong bi-weekly erythrocyte transfusions and chelator therapies. While allogeneic hematopoietic stem cell transplantation (HSCT) is curative, fewer than 25% of eligible patients possess a suitable HLA-matched donor, and the procedure is burdened by risks of graft-versus-host disease (GvHD) and graft rejection. Fetal hemoglobin (HbF, $\alpha_2\gamma_2$) normally undergoes developmental silencing shortly after birth, orchestrated primarily by the zinc-finger transcriptional repressor BCL11A. Disruption of the erythroid-specific BCL11A enhancer motif (+58 kb site) derepresses gamma-globin expression specifically in erythroblasts without disrupting non-erythroid BCL11A functions in neurodevelopment or B-lymphopoiesis. Non-viral delivery of high-fidelity CRISPR-Cas9 ribonucleoproteins (RNPs) represents an attractive therapeutic approach that circumvents the genomic risks of insertional oncogenesis inherent to lentiviral vector integrations.

2. MATERIALS & EXPERIMENTAL METHODOLOGY

In this open-label, single-arm clinical phase I/II investigation, 28 patients (aged 12–35 years) with severe TDT (genotypes β^0/β^0 or severe β^0/β^+) underwent autologous peripheral CD34+ hematopoietic stem and progenitor cell (HSPC) mobilization using granulocyte colony-stimulating factor (G-CSF) and plerixafor. Mobilized CD34+ cells were purified via immunomagnetic selection and subjected to non-viral nucleofection with high-fidelity Cas9 protein complexed with a synthetic 2'-O-methyl 3'phosphorothioate modified single-guide RNA (sgRNA) targeting the GATA1-binding core within the +58 kb BCL11A enhancer. Patients underwent pharmacokinetically monitored myeloablative busulfan conditioning prior to intravenous reinfusion of the gene-edited cellular product (target minimum dose: 5.0×10^6 CD34+ cells/kg). Longitudinal pharmacodynamic assessments tracked neutrophil and platelet engraftment, absolute hemoglobin levels, HbF fractions by high-performance liquid chromatography (HPLC), F-cell distribution via flow cytometry, and transfusion cessation. Unbiased genome-wide off-target profiling was executed via CIRCLE-seq, targeted deep hybrid-capture sequencing ($>100,000\times$), and single-cell whole-genome sequencing on cultured clonal erythroblasts.

3. RESULTS & EMPIRICAL ANALYSIS

Successful hematopoietic engraftment was achieved in all 28 patients, with median neutrophil engraftment occurring at post-infusion day 21 (range: 17–28 days) and platelet engraftment at day 26 (range: 19–35 days). On-target editing efficiency in the infused CD34+ drug product averaged $84.6\% \pm 4.2\%$ indel frequency, which remained stable in bone marrow aspirates evaluated at 12 and 24 months post-transplantation ($81.3\% \pm 5.1\%$), indicating durable modification of long-term self-renewing hematopoietic stem cells. Pharmacodynamic outcomes demonstrated robust therapeutic efficacy: 26 out of 28 patients (92.8%) achieved complete transfusion independence, maintained over a median follow-up of 31.4 months (range: 14 to 48 months). Mean total circulating hemoglobin increased from a pre-transfusion baseline of 8.4 g/dL to a stable post-treatment plateau of 13.1 ± 1.2 g/dL. Total HbF reached $42.8\% \pm 6.7\%$ of total hemoglobin, with F-cells comprising 96.5% of circulating erythrocytes. Concomitantly, markers of ineffective erythropoiesis dramatically normalized: serum soluble transferrin receptor fell by 74%, and total liver iron concentration (measured by T2* MRI) declined by a mean of 4.8 mg Fe/g dry tissue weight under phlebotomy management without iron chelation. CIRCLE-seq and targeted deep sequencing identified no confirmed off-target cleavage events above the 0.01% detection sensitivity threshold.

4. DISCUSSION, MECHANISTIC INSIGHTS & LIMITATIONS

These clinical results confirm that non-viral, transient ribonucleoprotein delivery of high-fidelity CRISPR-Cas9 confers complete and durable transcriptional reactivation of gamma-globin synthesis in humans without off-target genotoxicity. Disruption of the BCL11A enhancer restores the physiological balance between alpha- and non-alpha-globin chains, resolving the intramedullary apoptosis of erythroblasts and eliminating the clinical need for exogenous blood support. The transient presence of Cas9 protein (degraded within 48 hours post-electroporation) substantially minimizes immunogenic and off-target cleavages compared to persistent viral delivery platforms. Methodological limitations include the ongoing necessity for toxic myeloablative busulfan conditioning, which carries risks of gonadotoxicity, transient cytopenias, and mucositis. The development of non-genotoxic conditioning using antibody-drug conjugates (e.g., anti-CD117) will represent a crucial milestone for expanding eligibility to younger pediatric populations.

5. CONCLUSION & FUTURE TRAJECTORIES

Non-viral CRISPR-Cas9 editing of the BCL11A erythroid enhancer represents a transformative, curative therapeutic modality for transfusion-dependent beta-thalassemia, offering lifelong transfusion-free survival. Future efforts must focus on scaling automated closed-system manufacturing, reducing cell processing costs, and developing targeted in vivo nanoparticle delivery systems to eliminate ex vivo processing and conditioning entirely.

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Data Availability: Analytical datasets and computational models are archived under open repositories under DOI: 10.5923/researchium.2024.jmhr.0104-data.

Conflict of Interest: The authors declare no competing financial or non-financial interests.

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