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Perspective

CRISPR-Loaded Lipid Nanoparticles for *In Vivo* Correction of Sickle Cell Disease Mutation in Hematopoietic Stem Cells

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DESCRIPTION

Sickle Cell Disease (SCD) results from a single point mutation in the β-globin gene, causing production of abnormal hemoglobin that polymerizes under deoxygenated conditions. While *in vivo* gene editing approaches have shown promise in clinical trials, they require complex harvesting, manipulation, and reinfusion procedures that limit accessibility. We have developed specialized Lipid Nanoparticles (LNPs) capable of delivering Clustered Regularly Interspaced Short Palindromic Repeats associated Protein 9 (CRISPR-Cas9) ribonucleoprotein complexes and donor DNA templates directly to Hematopoietic Stem and Progenitor Cells (HSPCs) *in vivo*, potentially enabling one-time systemic treatment for SCD. These LNPs were engineered with a novel ionizable lipid containing a hydroxylated head group and asymmetric hydrophobic tails that demonstrated superior stability in circulation while enabling efficient endosomal escape following cellular uptake.

The LNP formulation was optimized through iterative design and high-throughput screening, resulting in particles approximately 75nm in diameter with narrow polydispersity (PDI<0.1) and neutral surface charge under physiological conditions. Surface functionalization with CD117 (c-Kit) targeting antibody fragments enabled specific binding to HSPCs while minimizing uptake by non-target cells, particularly in the liver. Cryo-electron microscopy revealed multilamellar internal structure with uniform RNP complex distribution throughout the particle core. Encapsulation efficiency exceeded 90% for Cas9 protein and 85% for guide RNA and donor DNA template, with all components maintaining functional integrity following the manufacturing process, as confirmed by *in vitro* editing assays.

In vitro studies using human CD34+ HSPCs demonstrated approximately 63% specific editing at the β-globin locus with minimal off-target modifications (<0.1% at top predicted sites) as assessed by next-generation sequencing. Colony-forming assays confirmed maintenance of multi-lineage differentiation potential following treatment, with edited cells demonstrating normal proliferation kinetics and differentiation capacity. Importantly, corrected cells showed physiological hemoglobin production with normal oxygen affinity curves when differentiated into erythroid lineages, confirming functional correction of the disease phenotype at the protein level.

For *in vivo* evaluation, we utilized a humanized SCD mouse model expressing the human β-globin gene with the sickle mutation. Following optimization of conditioning regimens to enhance HSPC accessibility, intravenous administration of our targeted LNPs resulted in approximately 42% gene correction in bone marrow HSPCs as assessed by deep sequencing of isolated LinCD117+Sca1+ cells. This editing efficiency translated to approximately 38% corrected hemoglobin in circulating erythrocytes by 16 weeks post-treatment, with concomitant improvements in hematological parameters including increased red blood cell count, reduced reticulocyte percentage, and normalized cell morphology. Pathophysiological improvements included reduced vaso-occlusive episodes following hypoxic challenge, decreased tissue iron deposits, and improved spleen architecture.

CONCLUSION

Safety evaluations revealed transient cytokine elevation following administration that resolved within 72 hours, with no evidence of sustained inflammation or immune activation. Comprehensive biodistribution studies demonstrated minimal accumulation in off-target tissues beyond 48 hours post-injection. Importantly, long-term genotoxicity assessment through integration site analysis and whole-genome sequencing of bone marrow cells at 6 months post-treatment revealed no evidence of insertional mutagenesis or chromosomal abnormalities. The translational potential of this approach is supported by successful scale-up to production quantities suitable for large animal studies, with consistent physicochemical properties and functional performance across multiple

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manufacturing runs. This in vivo gene editing platform represents a significant advancement toward non-invasive, one-time treatments for SCD and potentially other monogenic hematological disorders affecting HSPCs.