Profound and Durable Silencing of PCSK9 in Non-Human Primates by Epigenome Modulation

Poster ID: 679

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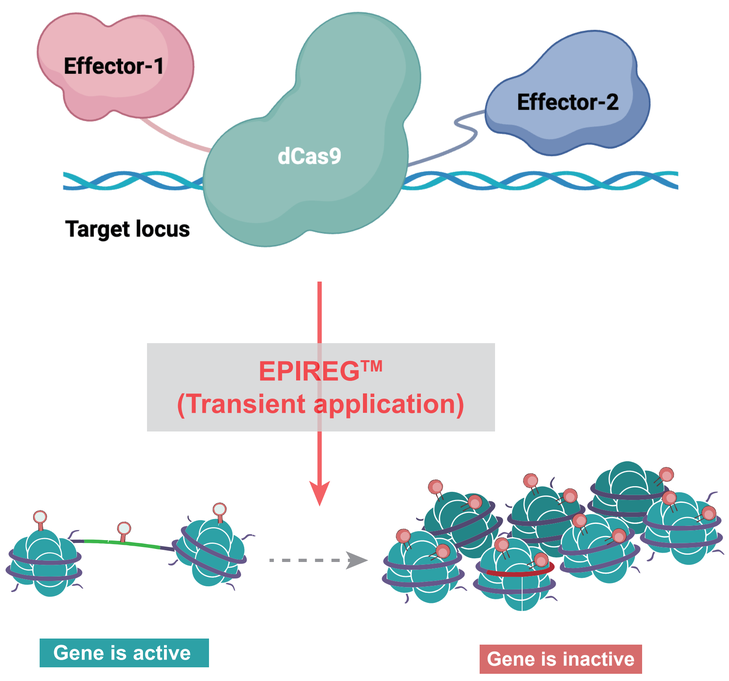
INTRODUCTION

Heterozygous Familial Hypercholesterolemia (HeFH) is an autosomal dominant genetic disorder precipitated by mutations in the LDL receptor gene, culminating in aberrantly elevated serum levels of low-density lipoprotein cholesterol (LDL-C). With a global prevalence ranging between 1 in 200 to 1 in 500, HeFH significantly augments the risk of atherosclerotic cardiovascular events. As such, the safety and specific silencing of the PCSK9 gene in hepatocytes using epigenetic modulation present as a potentially attractive next-generation treatment for HeFH, which may durably reduce the expression of PCSK9 without the risk of DNA cutting.

Objective: To progress the development of EPI-001, an epigenetic modulation therapeutic formulated as a lipid nanoparticle (LNP) targeting the human PCSK9 gene, specifically for HeFH treatment. EPI-001 is on track for clinical advancement, with an IND submission anticipated in 2024.

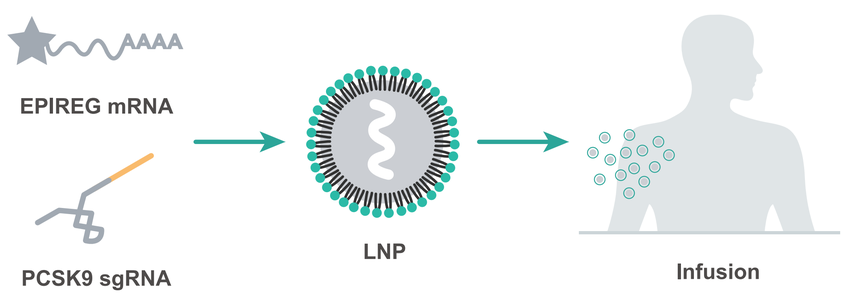
EPIREG™ Platform:



▪ *Durable change in phenotype without a change in genotype*

METHOD

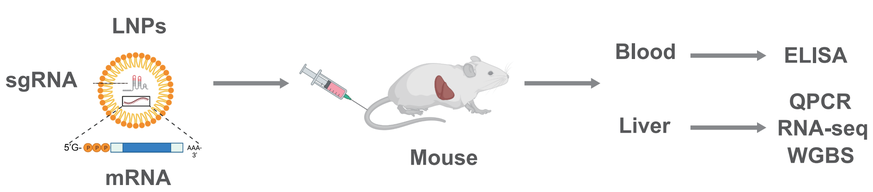
EPI-001 design



Key Advantages of LNP Delivery

- Large cargo capacity for EPIREG
- Transient expression
- Scalable synthetic manufacturing
- Redosing capability
- Low immunogenicity, well-tolerated, & biodegradable

RESULTS



Post-injection analysis:

Blood: PCSK9 protein levels were analyzed using the ELISA.

Liver:

- **PCSK9 expression was analyzed using qPCR.**
- **Potential off-target gene expression changes were studied using RNA-seq.**
- **Potential off-target DNA methylation changes were investigated using WGBS**

RESULTS

▪ Wild type mice

Significantly reduced Pcsk9 mRNA expression in the liver and its protein levels in the blood of treated mice, showing a dose-dependent effect.

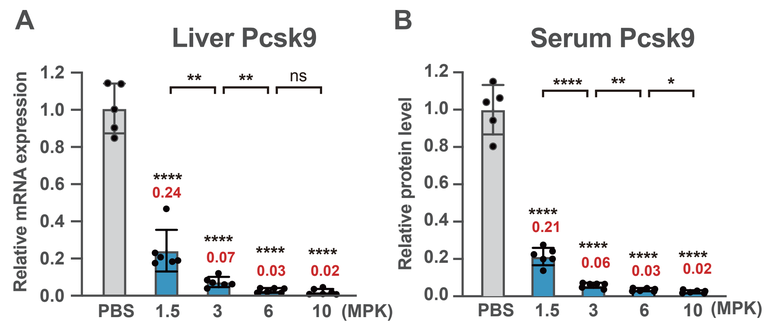


Figure 1: Efficient and inheritable silencing of Pcsk9 in mice. (A) The Pcsk9 mRNA levels in wild-type mouse liver, assessed one week after treatment with indicated doses (mg RNA per kg body weight). MPK, mg/kg. **(B)** Protein levels of Pcsk9 in the blood of mice treated with indicated doses. n = 5 for PBS, and n = 6 for LNP formulation groups.

▪ 70% Hepatectomy liver regeneration model

During the process of liver regeneration, hepatocytes undergo multiple rounds of proliferation.

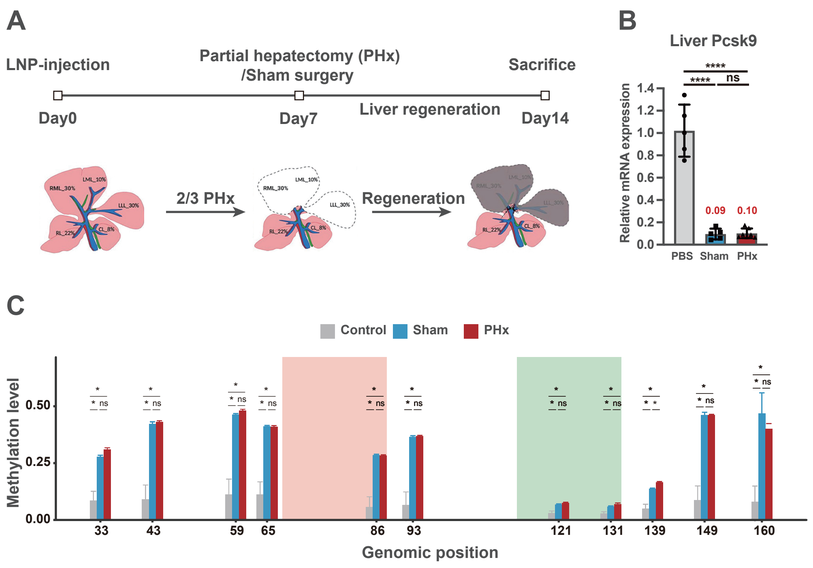
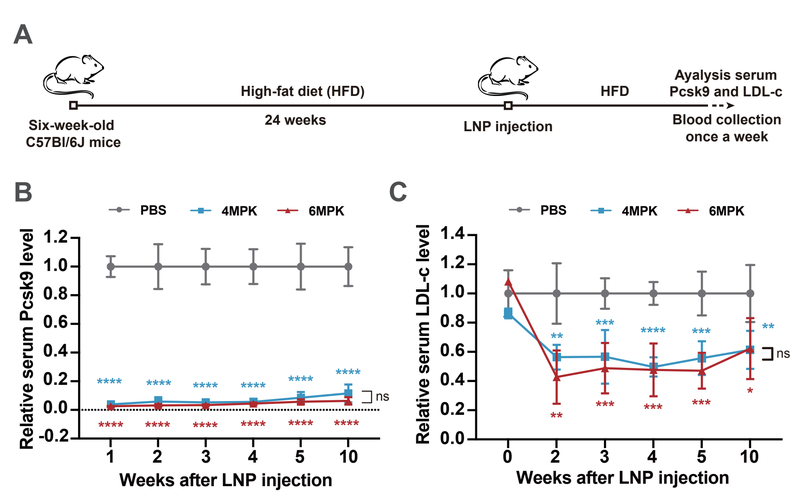


Figure 2: EPI-001 can efficiently inhibit the PCSK9 gene during liver regeneration and maintain stable methylation. (A) Diagram of the PHx experiment. **(B)** Comparison of liver Pcsk9 mRNA levels in mice at 7 days after PHx or Sham surgery. **(C)** Targeted bisulfite sequencing analysis of the CpG dinucleotides at promoter of the Pcsk9 gene. Red and green rectangles represent the targeting loci of sgRNAs. The methylation levels at each CpG dinucleotide were quantified by the averaged beta value from 4 or 3 biological replicates.

▪ High-fat diet (HFD) induced disease models

In a mouse model of a high-fat diet, sustained inhibition was observed for 10 weeks with a single dose administered



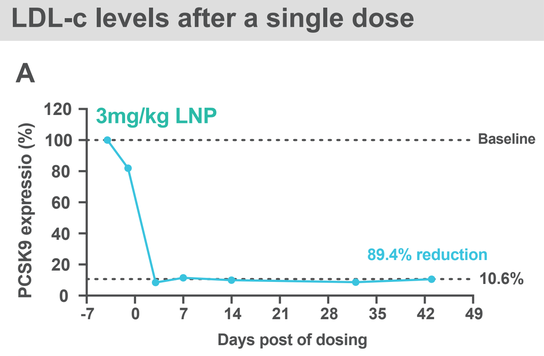
>95% serum PCSK9 reduction is maintained at 10 weeks

Approximately50% serum LDL-c reduction for 10 weeks

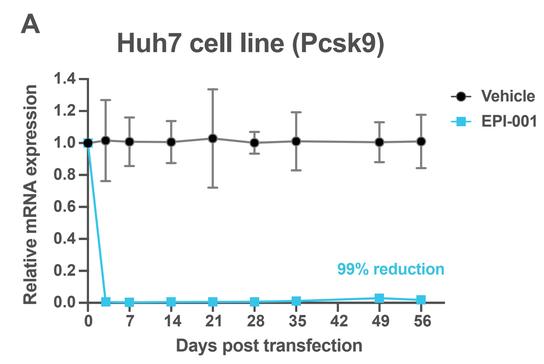
Figure 3: Lowering Pcsk9 expression in mice on HFD. (A) Diagram of Pcsk9 repression by epigenome editing in mice on HFD. **(B)** Protein levels of Pcsk9 in the serum of mice on HFD at 2 to 10 weeks after treatment with PBS, 4mg/kg or 6mg/kg LNP formulation with EPIREG mRNA and sgRNAs targeting Pcsk9. MPK, mg/kg. **(C)** Comparison of serum LDL-c levels in mice on HFD for 10 weeks after treatment.

▪ Non-human primate

Non-human primates (NHPs) achieve therapeutically relevant and sustained reductions in serum PCSK9 protein and LDL-c levels after a single dose



▪ hEPI-001 inhibitory activity



Single dose of EPI-001 lead to >50 Days durable repression of PCSK9 mRNA

Single dose of EPI-001 lead to >100 Days durable repression of serum PCSK9

Summary

- **EPIREG™ demonstrates deep and durable repression of PCSK9 in multiple species, including mouse and NHP, humanized PCSK9 mice.**
- **A single dose of a PCSK9-targeting epigenetic editor in NHP resulted in a 90% reduction in PCSK9 protein and a 56% reduction in LDL-C at least 45 days after treatment.**
- **Epigenetic editing represents a new class of therapeutics designed for life-long repression of PCSK9 without nicking.**

Acknowledgments

We thank the entire EpigenicTX team and our collaborators at CEBSIT.

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