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ARCUS gene editing of Apolipoprotein C3 results in substantial reduction in serum triglycerides *in vivo*

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INTRODUCTION

- Familial Chylomicronemia Syndrome (FCS) is a rare genetic disease that results in dangerously high levels of plasma triglycerides (TGs).
- Patients suffering from FCS are unable to correctly metabolize lipids, resulting in TG accumulation in the bloodstream and increased risk of pancreatitis.
- Apolipoprotein C3 (APOC3), a secreted glycoprotein synthesized primarily in the liver, is known to regulate plasma TG levels by interfering with hepatic clearance of TG-rich lipoproteins.
- APOC3 is associated with elevated plasma TG levels and cardiovascular disease risk, and patients with a naturally occurring null mutation in APOC3 exhibit cardioprotective effects.
- Lipid nanoparticles (LNPs) have been used clinically for delivery of both siRNA and mRNA for various therapeutics, including recently for the treatment of transthyretin amyloidosis via CRISPR/Cas9 gene editing.
- We propose disrupting APOC3 protein expression in the liver by delivering an mRNA-encoded ARCUS nuclease via LNP as a potential one-time curative treatment for patients with FCS.

WHAT IS ARCUS?

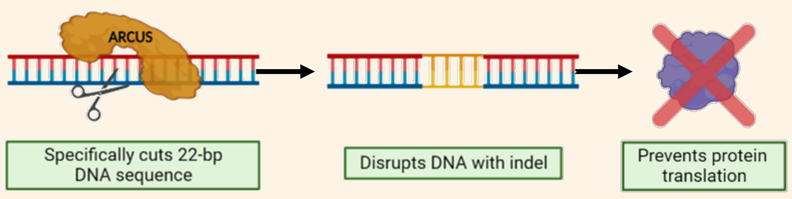


FIGURE 1. Mechanism of ARCUS gene disruption

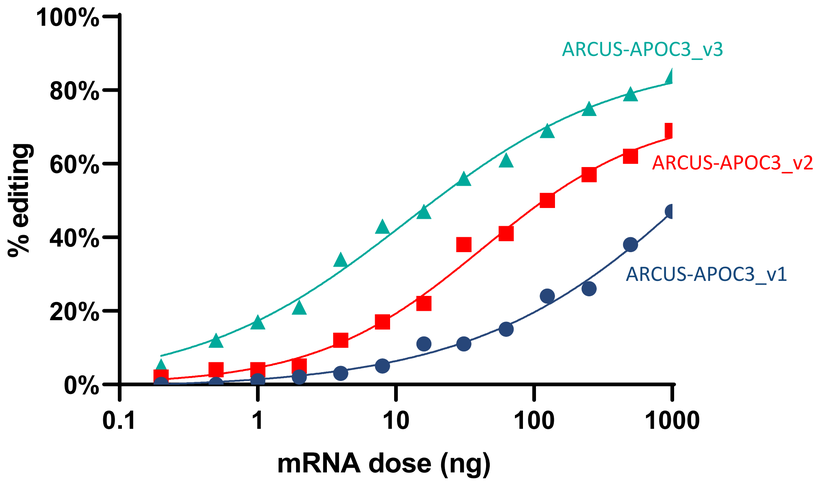
- ARCUS is a single-component protein derived from I-*Cre*I which contains both a 22 bp site-specific DNA recognition interface and endonuclease activity.
- The small size of ARCUS (364 aa) makes it easy to package into AAV or LNP for efficient delivery to affected cells.
- Multiple rounds of optimization are performed to increase both cutting efficiency and specificity with safety as the top priority.

OBJECTIVE

- To evaluate the feasibility of our therapeutic approach, we utilized APOC3 transgenic mice, which contain multiple copies of the human APOC3 (hAPOC3) gene and exhibit extremely high levels of circulating TGs.
- Following potency evaluation *in vitro*, we delivered two different ARCUS nucleases designed to target the hAPOC3 gene (ARCUS-APOC3_v2 and ARCUS-APOC3_v3) to hAPOC3 transgenic mice via systemic LNP administration.
- Study endpoints included transgene copy number, genomic editing (indels), hAPOC3 mRNA expression, hAPOC3 protein levels, and TG levels.

RESULTS

FIGURE 2. ARCUS-APOC3 nucleases demonstrate high on-target efficacy in HEK 293 cells, with increased activity following optimization

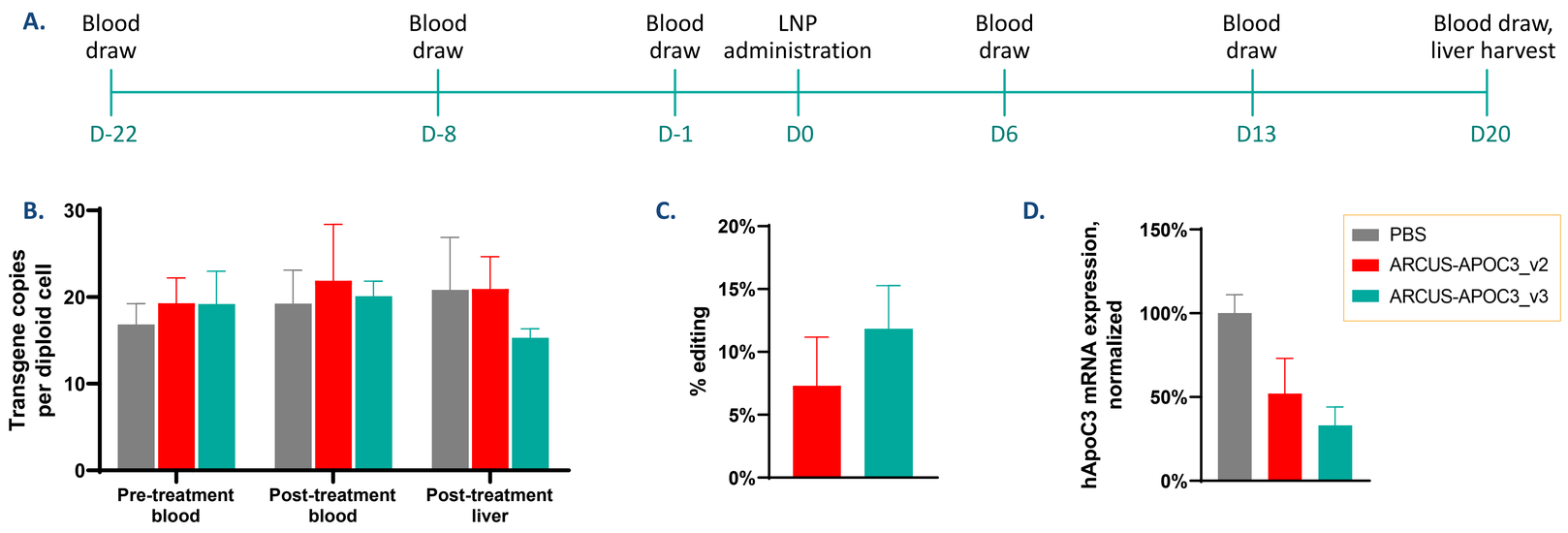


- Three generations of ARCUS-APOC3 nucleases were compared in HEK 293 cells for the ability to disrupt the APOC3 coding sequence. Various doses of each ARCUS-APOC3 mRNA were delivered to cells via RNA electroporation. Cellular DNA was isolated at day 2 post-transfection and percent editing was calculated using droplet digital PCR (ddPCR).
- On-target potency improved with each round of nuclease optimization. ARCUS-APOC3_v2 and ARCUS-APOC3_v3 were selected for further evaluated *in vivo.*

*[a]Statistics were calculated using an ordinary one-way ANOVA, Dunnett's multiple comparisons test (ns: P>0.05, *: P≤0.05, **: P≤0.01, ***: P≤0.001, ****: P≤0.0001).*

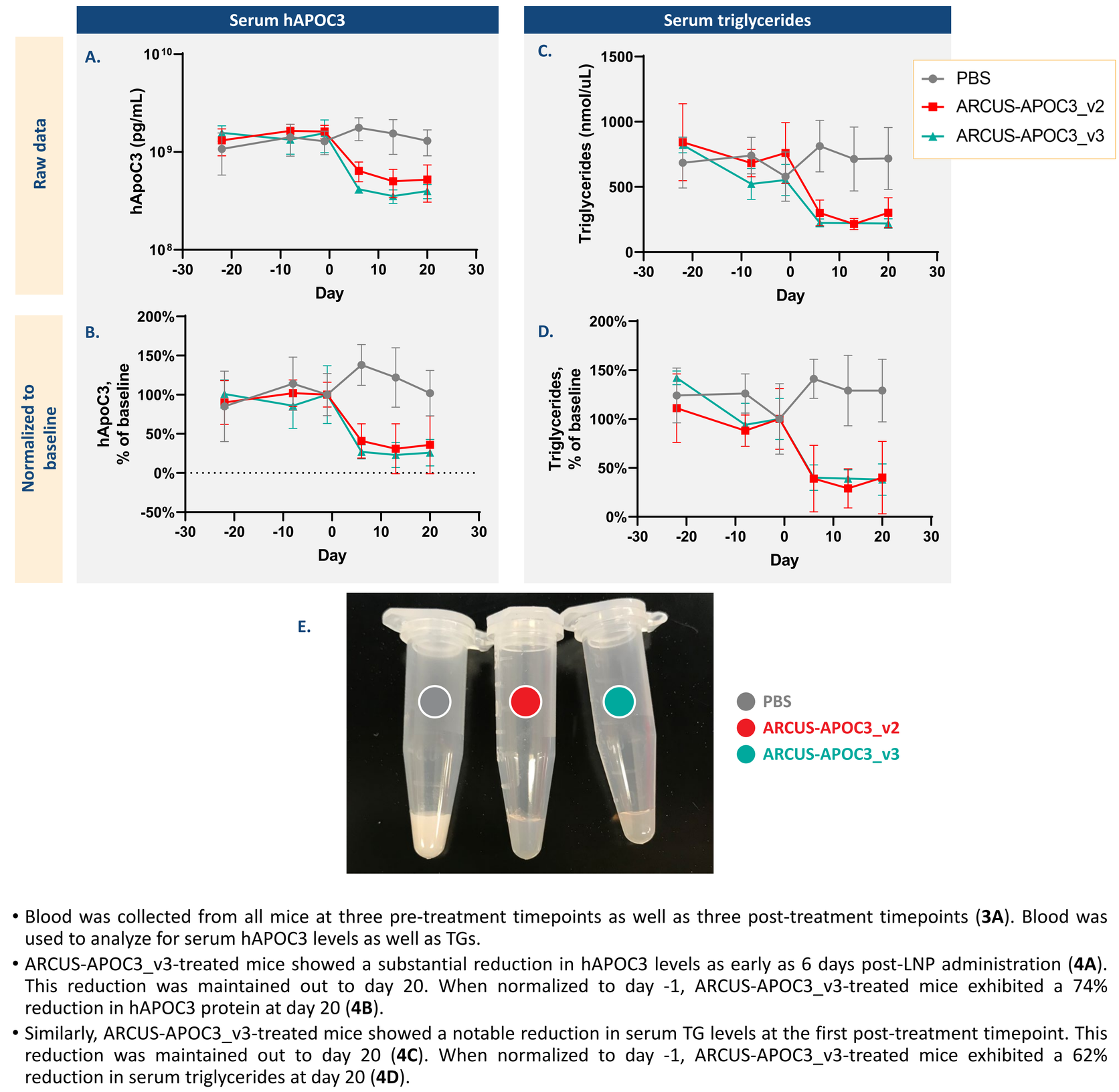
RESULTS (continued)

FIGURE 3. ARCUS-APOC3 nucleases successfully disrupt the hAPOC3 gene in transgenic mice, without significant loss of transgene copies



- ARCUS-APOC3_v2 and v3 were delivered retro-orbitally to hAPOC3 transgenic mice via LNP administration at a dose of 2 mg/kg. Mice were humanely euthanized for liver gDNA isolation at day 20 (**3A**).
- Transgene copy number was quantitated by ddPCR from the blood at both pre-treatment (D-22) and post-treatment (D20), as well as from the liver at D20. No significant changes in copy number were observed (**3B**).[a]
- Genomic editing in the liver was calculated by ddPCR. Similar to *in vitro*, ARCUS-APOC3_v3 was more potent than v2 and produced an average indel percentage of 11.84% ± 3.44% (**3C**).
- APOC3 mRNA expression in the liver was measured by ddPCR and normalized to GAPDH. ARCUS-APOC3_v3 gene editing resulted in a 67% reduction in hAPOC3 mRNA (**3D**).

FIGURE 4. ARCUS gene editing of hAPOC3 in transgenic mice results in substantial reductions in serum hAPOC3 and triglycerides



- Blood was collected from all mice at three pre-treatment timepoints as well as three post-treatment timepoints (**3A**). Blood was used to analyze for serum hAPOC3 levels as well as TGs.
- ARCUS-APOC3_v3-treated mice showed a substantial reduction in hAPOC3 levels as early as 6 days post-LNP administration (**4A**). This reduction was maintained out to day 20. When normalized to day -1, ARCUS-APOC3_v3-treated mice exhibited a 74% reduction in hAPOC3 protein at day 20 (**4B**).
- Similarly, ARCUS-APOC3_v3-treated mice showed a notable reduction in serum TG levels at the first post-treatment timepoint. This reduction was maintained out to day 20 (**4C**). When normalized to day -1, ARCUS-APOC3_v3-treated mice exhibited a 62% reduction in serum triglycerides at day 20 (**4D**).
- Serum isolated from one representative mouse in each cohort was photographed at day 6 post-LNP administration (**4E**), visualizing the stark reduction in circulating TGs seen in the ARCUS-treated animals.

CONCLUSIONS

1. ARCUS-APOC3 gene disruption in hAPOC3 transgenic mice results in potent reduction in hAPOC3 mRNA expression, serum hAPOC3, and serum triglyceride levels.
2. LNP-administration of ARCUS was well tolerated and generates rapid and detectable phenotypic changes.
3. Together, these data support the development of an LNP-administered ARCUS-APOC3 nuclease for the treatment of FCS.

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