CRISPR-Based *in vivo* Gene Editing Using an Engineered CasPhi Nuclease for Durable Reduction of apoC-III Protein and Triglycerides to Treat Persistent Chylomicronemia

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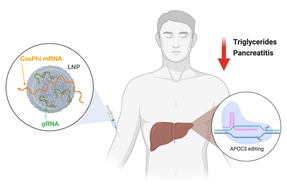
Mammoth Biosciences

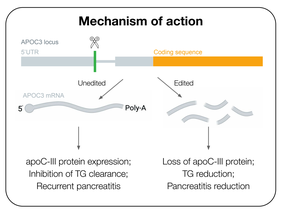
Introduction

Persistent Chylomicronemia (PC), including Familial Chylomicronemia Syndrome (FCS) and Multifactorial Chylomicronemia Syndrome (MCS), is a severe, life-threatening metabolic disorder characterized by extremely elevated fasting triglyceride (TG) levels routinely above 1,000 mg/dL, refractory to conventional lipid-lowering therapies. The most critical complication is recurrent acute pancreatitis (AP), which can carry a per-incidence mortality rate ranging from 5% to 30%.

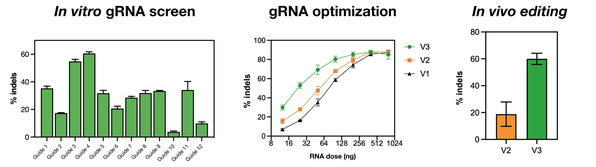
Apolipoprotein C-III (apoC-III, encoded by the *APOC3* gene), a protein expressed predominantly in the liver, is a key regulator of plasma TG levels by inhibiting lipoprotein lipase (LPL) and delaying the clearance of triglyceride-rich lipoproteins (TRLs). Human genetics data show that *APOC3* loss-of-function mutations are associated with reduced TGs and a lower risk of atherosclerotic cardiovascular disease (ASCVD). While existing *APOC3*-targeting antisense oligonucleotide (ASO) and RNA interference (RNAi) treatments have demonstrated efficacy in controlling TG levels, they necessitate repeated administration. A CRISPR-based gene editing approach, in contrast, offers the potential for durable therapeutic effect with a single-dose intravenous administration.

We developed MB-111 to genetically ablate *APOC3* using an engineered CasPhi nuclease, delivered as a mRNA together with a guide RNA (gRNA) encapsulated within a lipid nanoparticle (LNP) for targeted delivery to hepatocytes. Preclinical studies demonstrated robust activity: in a humanized transgenic mouse model of severe hypertriglyceridemia, administration of MB-111 led to a dose-dependent reduction in apoC-III protein and serum TGs. In nonhuman primates (NHPs), MB-111 achieved saturating liver editing and durable suppression of serum apoC-III that was sustained for nine months. MB-111 was generally well tolerated in NHPs, with only transient elevations in liver function tests which resolved within one week. These preclinical data support our plans for a first-in-human gene editing trial for the treatment of persistent chylomicronemia consisting of a multicenter dose escalation study to evaluate safety, tolerability, and efficacy of MB-111.



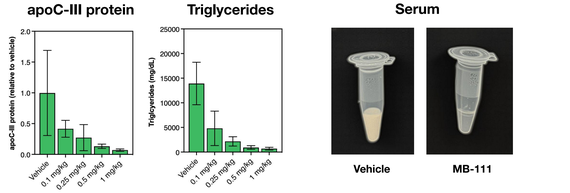


In vitro gRNA screening and optimization



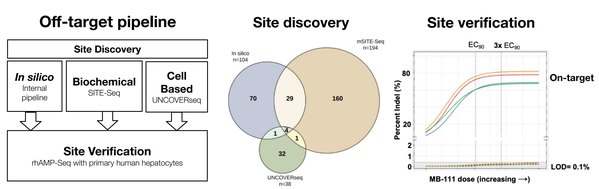
Screening and optimization of CasPhi gRNAs in primary human hepatocytes and in humanized *APOC3* mice. gRNAs were transfected alongside CasPhi mRNA by Lipofectamine MessengerMAX into primary human hepatocytes (subset of gRNAs shown). Iterative sequence engineering and chemical modification of the gRNA direct repeat resulted in increased activity in primary human hepatocytes and in mice harboring a humanized *APOC3* gene.

Efficacy in humanized hypertriglyceridemic mice



Treatment of Hypertriglyceridemic humanized *APOC3* mice with MB-111 surrogate reduces serum apoC-III protein and triglycerides. Humanized *APOC3* transgenic mice exhibit severely elevated triglyceride levels with lactescent serum reminiscent of persistent chylomicronemia. Dosing with lead gRNA and CasPhi mRNA using a surrogate LNP reduces serum apoC-III protein and triglycerides by up to 95%, resulting in amelioration of lactescent serum appearance.

Off-target and genotoxicity

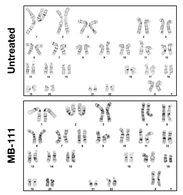


Off-target analysis in primary human hepatocytes. Potential off-target sites were identified using *in silico* prediction, Selective Enrichment and Identification of Adapter-Tagged DNA Ends by Sequencing (SITE-Seq), and Unbiased Nomination of CRISPR Off-target Variants using Enhanced RhPCR (UNCOVERseq). 297 nominated sites were assessed using amplicon sequencing of MB-111 treated primary human hepatocytes. A single dose-responsive off-target site was identified near limit of detection within an intronic region of the SNX18 gene. The site is 7,195 bp away from the nearest exon with no active regulatory elements overlapping the site in relevant cell types, and editing of this site does not alter SNX18 expression in hepatocytes (data not shown).

Summary of genome integrity assays

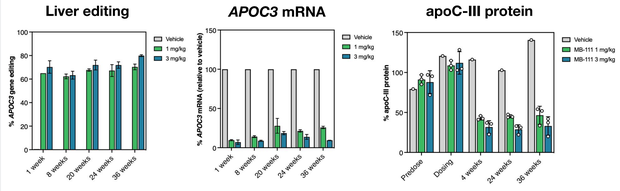
Assay	Resolution	Outcome
G-banding (karyotype)	Chromosome-level (Mb)	No treatment-related events
Optical Genome Mapping	Structural variants ≥500 bp	No novel treatment-associated SVs
Whole Genome Sequencing	Nucleotide-level	No treatment-related structural changes
rhAmp-Seq translocation	APOC3 ↔ OT-1 junction	Junction reads at background level
dPCR	APOC3 ↔ OT-1	No significant CNV

G-banding (karyotype)



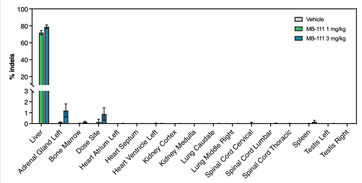
Genome structural integrity analysis in primary human hepatocytes. Macroscopic structural stability and ploidy were evaluated using G-banding (representative karyogram shown). Large structural variants across the whole genome were assessed using optical genome mapping (OGM). Nucleotide-resolution structural rearrangements were assessed by whole genome sequencing. Targeted assessment of potential translocations were performed using RNase H2-dependent Amplicon Sequencing (rhAmp-Seq) and digital PCR (dPCR). Collectively, no large-scale structural rearrangements or chromosomal instability was detected by these orthogonal assays.

Efficacy, biodistribution, and tolerability in NHPs

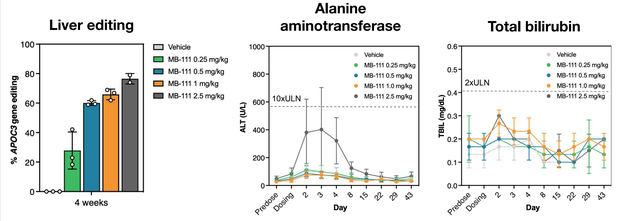


9-month durability of apoC-III protein reduction NHPs treated with MB-111. Non-human primates (NHPs, *M. fascicularis*) were administered with MB-111 at 1 mg/kg and 3 mg/kg doses. Both dose levels resulted in saturating liver editing, up to 90% mRNA reduction, and up to 67% serum apoC-III protein reduction that was sustained for 9-months post treatment (residual apoC-III derived from NHP intestinal expression).

Biodistribution



Tissue specificity of MB-111 in NHPs. At 1 mg/kg, editing was undetectable or near LOD across 16 non-liver tissues. At 3 mg/kg, low level editing was detected in adrenal gland, spleen, and dose site. No detectable editing was observed in any male or female reproductive tissues. No germline editing was detected in a germline transmission study (not shown).

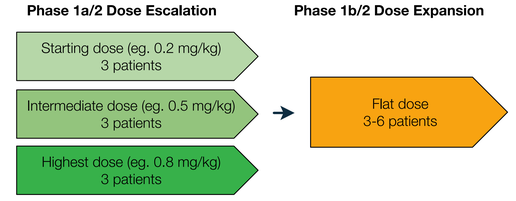


Dose Range Finding and Tolerability of MB-111 in NHPs. NHPs were dosed at 0.25, 0.5, 1.0, and 2.5 mg/kg. Near saturating editing was observed at ≥ 0.5 mg/kg dose level. No significant elevations in liver functional tests (LFTs) were observed up to 1 mg/kg. Transient LFT elevations were observed at the 2.5 mg/kg dose level not exceeding 10x ULN for alanine aminotransferase (ALT) or 2x ULN for bilirubin, which resolved in 1 week.

GLP Toxicology Findings

- There were no MB-111 LNP-related clinical observations, and no MB-111 LNP-related effects on appetence, body weights, electrocardiographic findings, respiratory rate, neurologic assessments, hematology and urinalysis parameters, and cytokine induction.
- There were no MB-111 LNP-related organ weight effects, or macroscopic and microscopic findings.
- Immunogenicity assessment showed limited anti-CasPhi nuclease antibody positivity in a small number of animals, with no detection of anti-PEG-2000-C-DMA antibodies.
- Transient, nonadverse coagulation and serum chemistry findings of decreased fibrinogen were observed at 2.5 mg/kg and increased liver enzyme activities (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyltransferase, and total bilirubin) were observed in few animals at ≥ 1.5 mg/kg
- The no-observed-adverse-effect-level (NOAEL) was deemed to be 2.5 mg/kg, the highest dose tested.

Planned Phase 1/2 Clinical Trial Design



Number of participants

- Up to ~18 total across 2 parts
- Adults of age 18-65 y/o
- Part A (dose escalation): 9-12 patients, 3+3 design, 3 dose levels are planned
- Part B (flat-dose expansion): 3-6 patients at flat dose converted from OBD

Endpoints

- **Primary:** safety/tolerability – TEAEs, AESIs, SAEs from Day1 to end of study
- **Secondary:** Δ apoC-III, Δ fasting TG; PK of CasPhi mRNA, gRNA, PEG & ionizable lipid
- **Exploratory**: confirmed pancreatic events, PANQOLI QoL, other lipids & hepatic fat fraction

Key inclusion criteria

- Persistent Chylomicronemia with fasting serum TGs ≥1000 mg/dL (≥11.3 mmol/L)
- Severe hypertriglyceridemia resistant to standard-of-care non-apoC-III targeting lipid lowering therapy

Key exclusion criteria

- Liver dysfunction / fibrosis; cytopenias; eGFR <45; uncontrolled diabetes or thyroid disease
- Cardiac: QTcF prolongation, LVEF <45%, significant stenosis; HIV/HBV/HCV positive
- Recent apoC-III therapy (volanesorsen / olezarsen ≤ 90d; plozasiran ≤ 365d); prior gene therapy or gene editing therapy
- Pregnancy or lactation; PEG hypersensitivity, recent alcohol/substance abuse

Conclusions

MB-111 is an *in vivo* gene editing therapy with the potential to durably treat persistent chylomicronemia by targeting *APOC3*

- MB-111 robustly reduces apoC-III and triglycerides in a mouse model of severe hypertriglyceridemia.
- MB-111 achieves durable suppression of apoC-III for at least 9 months in non-human primates.
- MB-111 is well tolerated in NHPs with a wide therapeutic window.
- MB-111 exhibits a favorable genotox profile, highly specific liver biodistribution, and no detectable germline editing.
- A multi-center Phase 1/2 clinical trial is being initiated to evaluate safety and efficacy of MB-111.

Acknowledgements

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