Pharmacokinetics and pharmacodynamics of EDIT-401(mu), *in vivo* gene-editing therapy for lowering LDL-C in mice

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Introduction

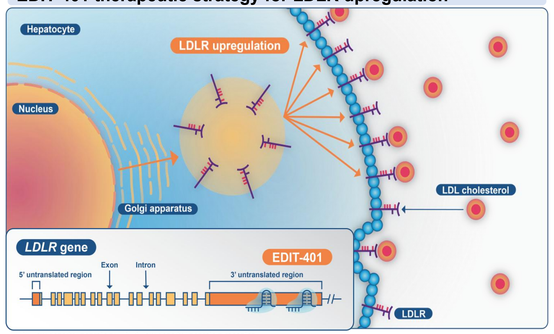
- Heterozygous familial hypercholesterolemia (HeFH) is a genetic disease caused primarily by autosomal dominant, loss-of-function mutations in the low-density lipoprotein receptor (*LDLR*) gene, leading to severely elevated low-density lipoprotein cholesterol (LDL-C) levels and increased risk of atherosclerotic cardiovascular disease.
- Despite maximally tolerated standard of care treatment, patients with HeFH often fail to achieve target LDL-C levels, warranting development of a transformative LDL-C–lowering therapy.
- EDIT-401, a single-dose, experimental, *in vivo*, clustered regularly interspaced short palindromic repeats (CRISPR)-based gene-editing medicine, currently in preclinical development, edits the *LDLR* gene to increase LDLR protein expression on hepatocyte cell surface and consequently reduces serum LDL-C levels.
- The nonclinical studies were designed to evaluate pharmacokinetics (PK) and pharmacodynamics (PD) of EDIT-401(mu), EDIT-401 containing murine surrogate guide RNAs (gRNAs), in wild-type (WT) C57BL/6J and heterozygous murine low-density lipoprotein receptor (*Ldlr*) knockout (*Ldlr*$^{+/-}$) C57BL/6J mice. *Ldlr*$^{+/-}$ knockout mice harbor a loss-of-function mutation in a single *Ldlr* allele, modelling patients with HeFH, and have an elevated level of LDL-C compared with WT mice. This study enables us to evaluate whether human dose predicted based on non-human primate (NHP) data, needs to be adjusted for patients with HeFH.

Methods

EDIT-401 is an N-acetylgalactosamine (GalNAc)-based liver-targeting lipid nanoparticle (LNP) (licensed from Genevant Sciences) carrying CRISPR-associated protein 9 (Cas9) mRNA and two gRNAs targeting the 3′ untranslated region of *LDLR* gene. PK and PD of EDIT-401(mu) were evaluated in WT and *Ldlr*$^{+/-}$ mice following a single intravenous bolus administration across multiple dose levels. Both WT and *Ldlr*$^{+/-}$ mice (n=4 per dose group) received single dose ranging from 0.05 to 1 mg/kg. Following EDIT-401(mu) dosing, sparse plasma samples were collected for PK analysis, with Cas9 mRNA measured as a proxy for EDIT-401(mu) by reverse transcription droplet digital polymerase chain reaction (RT-ddPCR). Liver samples were also collected up to 14 days post-dose to assess Cas9 mRNA exposure, editing, and LDLR protein upregulation. Serum samples were collected up to 14 days post-dose to measure LDL-C levels by high-performance liquid chromatography (HPLC). LDLR protein was measured by enzyme-linked immunosorbent assay (ELISA). PK parameters were derived using non-compartmental analysis (NCA) with Phoenix WinNonlin software and are reported as mean values for all the tested doses of EDIT-401(mu). LDL-C reduction and LDLR fold-upregulation were analyzed using non-linear fit with GraphPad Prism (V10.4.0).

EDIT-401 uses a differentiated mechanism of action to reduce LDL-C

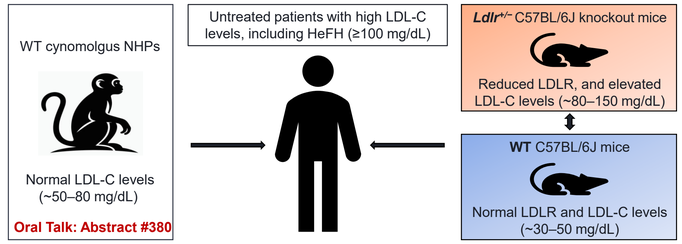
EDIT-401 therapeutic strategy for LDLR upregulation



LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor.

- CRISPR/Cas9 nuclease and dual gRNAs disrupt negative regulatory elements of the *LDLR* gene, increasing mRNA stability, enabling LDLR protein upregulation.
- This amplification approach enables ≥90% mean reduction in serum LDL-C in NHP and mouse models.

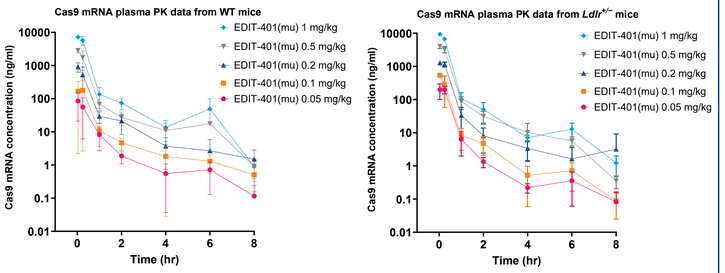
Figure 1: Elevated levels of LDL-C observed in heterozygous loss-of-function (*Ldlr*$^{+/-}$) mice modeling HeFH



Reference for *Ldlr*$^{+/-}$ homozygous mice: https://www.jax.org/strain/002207. HeFH, heterozygous familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; *Ldlr*, murine low-density lipoprotein receptor; NHP, non-human primate; WT, wild-type.

Ldlr$^{+/-}$ C57BL/6J mice were generated by inserting a neomycin resistance cassette at exon 4 leading to the disruption of *Ldlr* coding DNA sequence. *Ldlr*$^{+/-}$ C57BL/6J mice have one intact *Ldlr* allele and one mutated *Ldlr* allele, generated by crossing WT mice with *Ldlr*$^{-/-}$ mice. The basal levels of LDL-C in *Ldlr*$^{+/-}$ mice is higher compared with control WT C57BL/6J mice.

Figure 2: EDIT-401(mu) exposure in systemic circulation is transient and increases with dose in both *Ldlr*$^{+/-}$ mice and WT mice



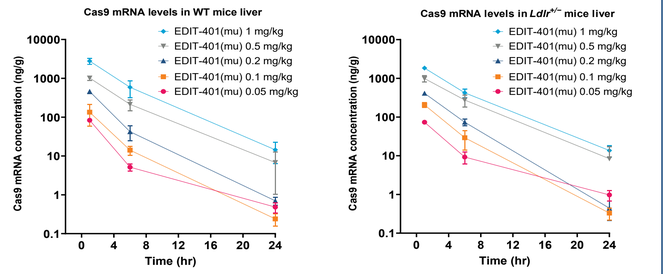
PK parameters	0.05 mg/kg	0.1 mg/kg	0.2 mg/kg	0.5 mg/kg	1 mg/kg
C_{max} (ng/mL)	85.8	179	919	2850	7260
$AUC_{0-\infty}$(hr*ng/mL)	45.8	110	380	1120	3060

PK parameters	0.05 mg/kg	0.1 mg/kg	0.2 mg/kg	0.5 mg/kg	1 mg/kg
C_{max} (ng/mL)	199	537	1270	4030	9430
$AUC_{0-\infty}$(hr*ng/mL)	98.2	178	597	1710	3410

AUC, area under the curve; Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats; C_{max}, maximum concentration; hr, hour; *Ldlr*, murine low-density lipoprotein receptor; PK, pharmacokinetic; WT, wild-type.

Total RNA was extracted from the collected sparse plasma samples and the levels of Cas9 mRNA were quantified by RT-ddPCR. Cas9 mRNA data was used to perform NCA using Phoenix WinNonlin.

Figure 3: Comparable dose-dependent liver exposure of EDIT-401(mu) observed between WT and *Ldlr*$^{+/-}$ mice



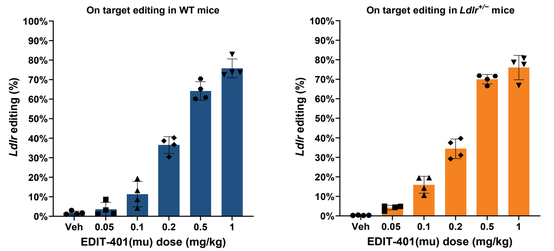
PK parameters	0.05 mg/kg	0.1 mg/kg	0.2 mg/kg	0.5 mg/kg	1 mg/kg
C_{max} (ng/g)	83.4	134	459	1020	2780
$AUC_{0-\infty}$(hr*ng/g)	290	497	1650	4890	13200
$t_{1/2}$(hr)	3.48	2.61	2.60	3.28	3.11

PK parameters	0.05 mg/kg	0.1 mg/kg	0.2 mg/kg	0.5 mg/kg	1 mg/kg
C_{max} (ng/g)	73.7	206	410	989	1840
$AUC_{0-\infty}$(hr*ng/g)	318	819	1730	5330	10700
$t_{1/2}$(hr)	4.05	2.50	2.36	3.40	3.34

AUC, area under the curve; Cas9, CRISPR-associated protein 9; C_{max}, maximum concentration; CRISPR, clustered regularly interspaced short palindromic repeats; hr, hour; *Ldlr*, murine low-density lipoprotein receptor; PK, pharmacokinetic; $t_{1/2}$,half-life; WT, wild-type.

Total RNA was extracted from liver samples collected at 1, 6, 24, 168, and 336 hours and the levels of Cas9 mRNA were quantified by RT-ddPCR. Cas9 mRNA levels were below limit of quantification at 168 and 336 hours post-dosing. Cas9 mRNA data was used to perform NCA using Phoenix WinNonlin.

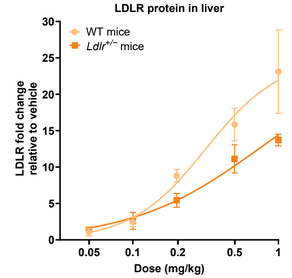
Figure 4: Comparable total editing observed between WT and *Ldlr*$^{+/-}$ mice



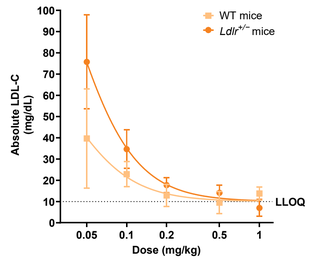
Reference for UDiTaS: 10.1186/s12864-018-4561-9. *Ldlr*, murine low-density lipoprotein receptor; UDiTaS, Uni-Directional Targeted Sequencing; Veh, vehicle; WT, wild-type.

Genomic DNA was extracted from liver samples collected at 336 hours post-dose. Percentage of total editing at the *Ldlr*$^{+/-}$ locus was then assessed by Uni-Directional Targeted Sequencing (UDiTaS).

Figure 5: Comparable LDL-C reduction between WT and *Ldlr*$^{+/-}$ mice with dose-dependent upregulation of LDLR



Mice strain	E_{max} LDLR fold change mean (± SD)	ED_{50} mg/kg mean (95% CI)
WT mice	23.1 (4.95)	0.31 (0.26–0.39)
Ldlr$^{+/-}$ mice	13.7 (0.71)	0.27 (0.23–0.31)



Mice strain	ED_{50} mg/kg
WT mice	0.04281
Ldlr$^{+/-}$ mice	0.03445

The slope of the two DRC curves were compared using extra sum-of-squares F test, and the difference in ED_{50} was not statistically significant, with a *P*-value of 0.848. The bottom of the curve was constrained to 10 mg/dL and shared Hill slope was applied across datasets. CI, confidence interval; DRC, dose response curve; ED_{50}, median effective dose; E_{max}, maximum effect; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; *Ldlr*, murine low-density lipoprotein receptor; LLOQ, lower limit of quantification; SD, standard deviation; WT, wild-type.

At 336 hours post-dose, levels of LDLR protein were analyzed from liver samples by ELISA, and LDL-C levels were analyzed in serum samples using HPLC.

Conclusions

- Preclinical PK findings demonstrate rapid uptake of EDIT-401(mu) from the systemic circulation into the liver in both WT and *Ldlr*$^{+/-}$ mice.
- Comparable and dose-dependent increase in total editing observed in both WT and *Ldlr*$^{+/-}$ mice.
- Rapid and robust decrease in LDL-C levels with similar reductions observed between WT and *Ldlr*$^{+/-}$ mice.
- Mouse PK/PD data demonstrate that EDIT-401 dose adjustments (estimated based on WT NHP data) may not be needed in patients with HeFH.

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