In vivo CRISPR-based upregulation of a compensatory pathway for normalization of phenotypes of a disease-causing gene

Poster #2324

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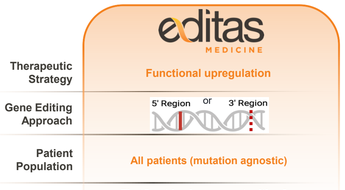
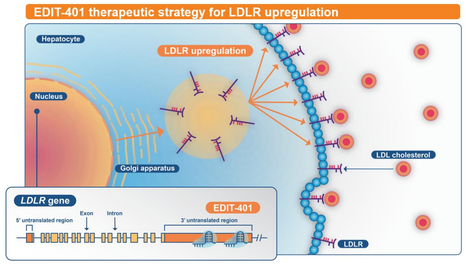
Introduction: Editas' differentiated *in vivo* gene editing upregulation strategy aims to deliver best-in-class potentially curative medicines for genetic diseases

By editing non-coding gene regulatory regions, such as 5' and 3' untranslated regions (UTRs), we seek to induce upregulation of wild-type alleles, functional paralogs, or compensatory pathways to mitigate diseases caused by pathogenic mutations:



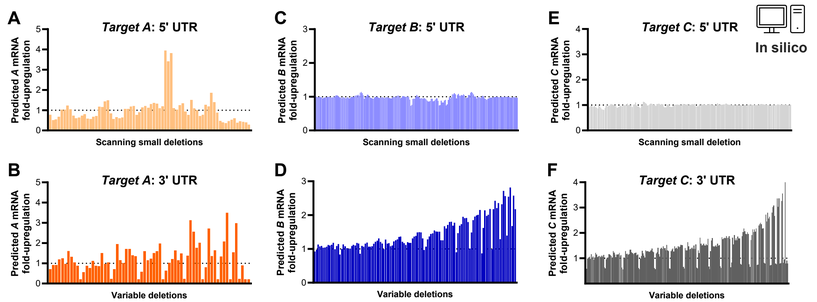


We harnessed the gene editing capabilities of *C*lustered *R*egularly *I*nterspaced *S*hort *P*alindromic *R*epeats (CRISPR) to develop EDIT-401 – an investigational therapeutic designed to upregulate the expression of liver LDLR and reduce LDL-cholesterol for individuals with heterozygous familial hypercholesterolemia at risk of experiencing a major cardiovascular event.

Guided by DNA large language models (DNA-LLMs), we build upon this validated CRISPR-based strategy to pursue the development of multiple novel *in vivo* therapeutics that upregulate genes-of-interest *Target A*, *Target B*, and *Target C* to compensate for loss-of-function mutations that cause monogenic diseases with severe unmet needs.

Figure 1: A DNA-LLM identified purported cis repressor elements within the non-coding regions of multiple genes-of-interest

Using a DNA-LLM, we performed comprehensive *in silico* scanning mutagenesis of the 5' and 3' UTRs of target genes *A*, *B*, and *C*:

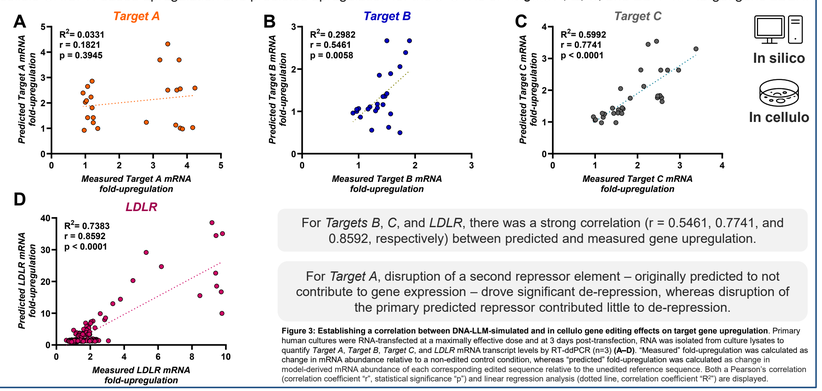


For all three gene targets, disruption of purported 5' and 3' UTR repressor were predicted to de-repress gene expression ~3- to 4-fold

Figure 1: DNA-LLM-based prediction of *Target A*, *B*, and *C* regulatory region disruption and the impact on their mRNA expression. Variable genomic editing outcomes were simulated *in silico*, and the resulting edited DNA sequences were provided as input to a sequence-to-function DNA-LLM prediction model to infer regulatory effects of gene editing. For 5' UTRs, 10 bp deletions with a 5 bp sliding window from the beginning of the annotated promoter to the coding sequence start codon were simulated **(A, C, E)**, while for 3' UTRs, deletions of variable size and position between the coding sequence stop codon and consensus polyA signal were simulated **(B, D, F)**. Predicted gene expression changes were quantified by comparing model-derived expression profiles over annotated exon regions for each edited sequence relative to the unedited reference sequence and expressed as fold-upregulation.

Figure 3: DNA-LLM-simulated gene editing is highly predictive of resulting target gene expression in relevant biological systems

To assess the accuracy of our DNA-LLM prediction modeling, we determined the correlations between the empirically-determined in cellulo CRISPR-based upregulation and predicted upregulation for the 3' UTRs of *Targets A*, *B*, *C*, and EDIT-401 target gene *LDLR*:



For *Targets B*, *C*, and *LDLR*, there was a strong correlation (r = 0.5461, 0.7741, and 0.8592, respectively) between predicted and measured gene upregulation.

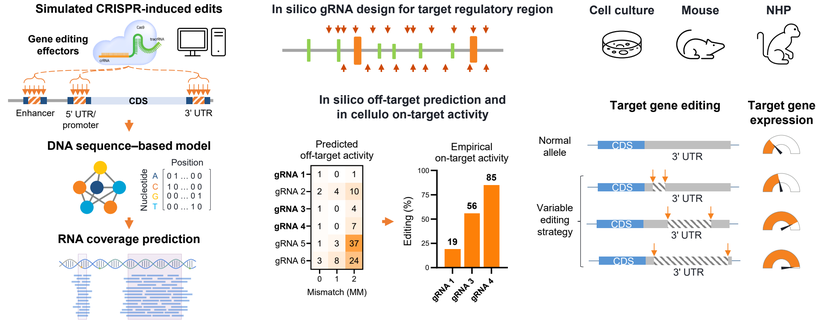
For *Target A*, disruption of a second repressor element – originally predicted to not contribute to gene expression – drove significant de-repression, whereas disruption of the primary predicted repressor contributed little to de-repression.

Figure 3: Establishing a correlation between DNA-LLM-simulated and in cellulo gene editing effects on target gene upregulation. Primary human cultures were RNA-transfected at a maximally effective dose and at 3 days post-transfection, RNA was isolated from culture lysates to quantify *Target A*, *Target B*, *Target C*, and *LDLR* mRNA transcript levels by RT-ddPCR (n=3) **(A–D)**. "Measured" fold-upregulation was calculated as change in mRNA abundance relative to a non-edited control condition, whereas "predicted" fold-upregulation was calculated as change in model-derived mRNA abundance of each corresponding edited sequence relative to the unedited reference sequence. Both a Pearson's correlation (correlation coefficient "r", statistical significance "p") and linear regression analysis (dotted line, correlation coefficient "R²") are displayed.

Methods: Workflow for designing and validating novel CRISPR-based strategies of gene upregulation

We use DNA-LLMs to delineate target gene repressor elements in non-coding regions, assess on- and off-target activity of a focused gRNA panel, and screen gene editing strategies combinatorially in relevant disease models to demonstrate target gene upregulation:

DNA-LLM-based prediction of target gene regulatory elements → **gRNA design and on- and off-target activity evaluation** → **Validation of gene upregulation strategy in cellulo and *in vivo***



Predicted increases in target mRNA abundance suggests the presence of negative regulatory elements

gRNA-nuclease combos with high predicted specificity are assessed for their on-target activity in cellulo

Specific/active effector combos are tested in relevant biological systems to identify top upregulation strategies

Figure 2: Informed gRNA mapping of target gene regulatory regions validated several editing strategies that induce robust gene upregulation

To develop novel CRISPR-based upregulation strategies, we combinatorially tested a panel of potent and specific gRNAs to disrupt DNA-LLM-predicted repressor elements within the UTRs of target genes *A*, *B*, and *C* and measured the impact on their mRNA expression in relevant primary human cell cultures:

Disruption of a single repressor element within the 5' UTR of *Target A* achieved only ~1.5-fold upregulation **(A)**.

3' UTR–targeting strategies for *Targets A*, *B*, and *C* exhibited robust upregulation on gene transcripts (~4-, ~2-, and ~3.5-fold upregulation, respectively) **(B–D)**.

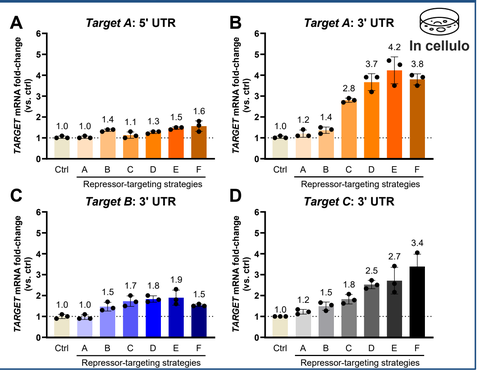
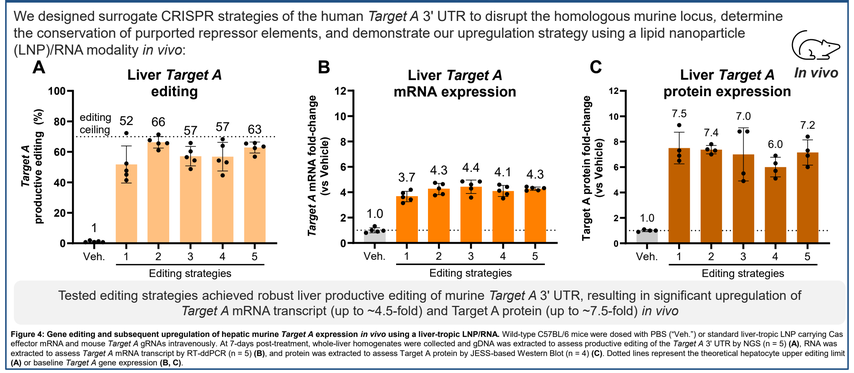


Figure 2: Screening of variable CRISPR cargos directed against different human *Target A*, *B*, and *C* gene regulatory regions-of-interest for their ability to induce gene expression in primary human cell cultures. Primary human cultures were RNA-transfected at a maximally effective dose and after 3 days post-transfection, RNA and gDNA were isolated from cell lysates to quantify target gene mRNA transcript levels by RT-ddPCR (n=3) **(A–D)**. mRNA abundances are reported relative to a non-edited "ctrl" condition with baseline levels represented by a dotted line.

Figure 4: Target A repressor elements are conserved in mouse, and their disruption induces hepatic gene upregulation *in vivo*

We designed surrogate CRISPR strategies of the human *Target A* 3' UTR to disrupt the homologous murine locus, determine the conservation of purported repressor elements, and demonstrate our upregulation strategy using a lipid nanoparticle (LNP)/RNA modality *in vivo*:



Tested editing strategies achieved robust liver productive editing of murine *Target A* 3' UTR, resulting in significant upregulation of *Target A* mRNA transcript (up to ~4.5-fold) and Target A protein (up to ~7.5-fold) *in vivo*

Figure 4: Gene editing and subsequent upregulation of hepatic murine *Target A* expression *in vivo* using a liver-tropic LNP/RNA. Wild-type C57BL/6 mice were dosed with PBS ("Veh.") or standard liver-tropic LNP carrying Cas effector mRNA and mouse *Target A* gRNAs intravenously. At 7-days post-treatment, whole-liver homogenates were collected and gDNA was extracted to assess productive editing of the *Target A* 3' UTR by NGS (n = 5) **(A)**, RNA was extracted to assess *Target A* mRNA transcript by RT-ddPCR (n = 5) **(B)**, and protein was extracted to assess Target A protein by JESS-based Western Blot (n = 4) **(C)**. Dotted lines represent the theoretical hepatocyte upper editing limit **(A)** or baseline *Target A* gene expression **(B, C)**.

Conclusions

- Utilizing a DNA-LLM, we established a cost-effective, accelerated workflow for developing *in vivo* CRISPR-based upregulation strategies for multiple target genes in parallel.
- Simulated mutagenesis and gene editing events proved sequence-to-function DNA-LLMs can be highly predictive of resulting mRNA expression, but with some limitations.
- Informed gRNA screening in relevant primary human cells confirmed repressor elements governing *Target A*, *B*, *C* expression and identified optimal upregulation strategies.
- We validated our methodologies *in vivo* by demonstrating robust liver *Target A* productive editing and mRNA and protein upregulation using LNP/RNA in mice.

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