In Vivo Gene Editing and Disease-Associated Biomarker Reduction for Multiple Liver Targets in Non-human Primate Using AsCas12a Nuclease Delivered by Lipid Nanoparticle Formulations

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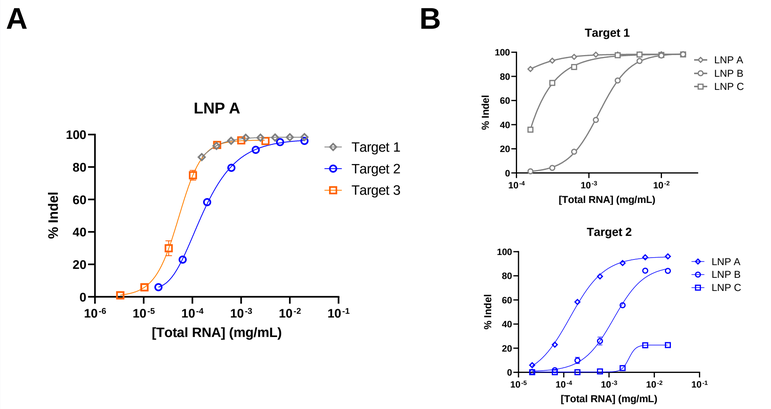
Introduction

- Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas gene editing is a transformative technology with the potential to enable durable treatments for many diseases with unmet medical needs.
- *Acidaminococcus* sp. Cas12a (AsCas12a) is a highly efficient and specific nuclease that targets a different protospacer adjacent motif sequence (TTTN) than Cas9, which could be advantageous for some targets.[1,2] To date, *in vivo* gene editing for liver targets using systemically delivered AsCas12a has not been demonstrated.
- Here we present proof-of-concept results from *in vivo* mouse and non-human primate (NHP) studies and show high levels of target gene editing in the liver and corresponding biomarker response following intravenous administration of AsCas12a mRNA and guide RNAs (gRNAs) delivered using lipid nanoparticles (LNPs). Using multiple different LNPs from a highly potent LNP platform, we demonstrate efficient target editing in the liver and reduction in serum protein biomarkers.
- We demonstrate the translatability of our approach by successfully demonstrating high levels of target gene editing and biomarker reduction across *in vitro* and *in vivo* preclinical models, including primary human hepatocytes, wild-type and transgenic mice, and NHPs.
- LNP biodistribution properties were assessed in NHPs using target gene editing and a green fluorescent protein (GFP) reporter mRNA. High levels of editing and GFP mRNA/protein were observed in NHP liver with minimal to no editing or GFP mRNA/protein detected in other non-target tissues, including the ovaries.
- These data highlight our ability to achieve high levels of *in vivo* liver gene editing and disease-associated biomarker reduction using a 'plug-n-play' approach that permits our novel AsCas12a nuclease mRNA to be combined with easily interchangeable target-specific gRNAs delivered with a proprietary LNP that has optimal properties for liver delivery to target diseases with unmet medical need.

Figure 1: Modular 'plug-n-play' approach *in vitro*

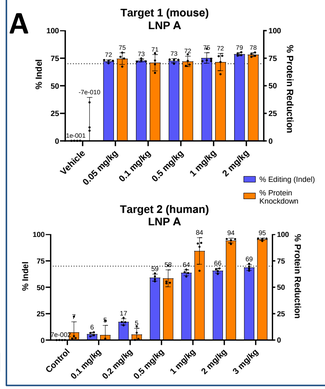
Proprietary gene editing platform enables a 'plug-n-play' approach to rapidly identify effective LNP:cargo combinations *in vitro*

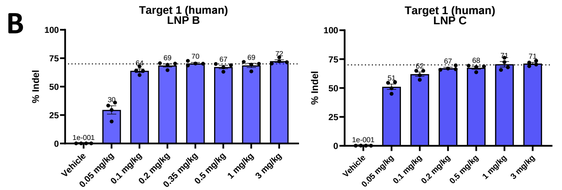


High levels of editing were observed in primary human hepatocytes (PHHs) across different targets **(A)** and LNPs **(B)** suggesting components can easily be interchanged to optimize editing and broaden target selection. PHHs were treated with different concentrations of liver-tropic LNPs carrying AsCas12a mRNA paired with different gRNAs and harvested 72 hours after transfection. Genomic DNA was extracted from cell pellets and editing at the target locus was then assessed by next-generation sequencing (NGS) to determine the frequency of indels. Transfections included recombinant human apolipoprotein E within cell culture media.

Figure 2: *In vivo* gene editing proof-of-concept demonstrated in mouse models

High *in vivo* liver editing and biomarker reduction in transgenic mouse models with LNP:cargo combinations demonstrates *in vivo* proof-of-concept of 'plug-n-play' approach

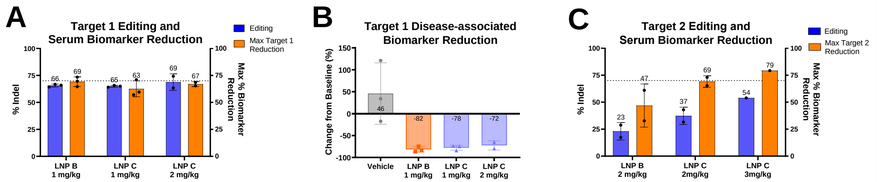




Wild-type or commercially available transgenic mice were dosed intravenously with LNPs. High levels of liver editing was observed in mouse experiments with two different gRNAs using a reference LNP (**A**). Maximal liver editing was observed in transgenic mice at low doses using Target 1 gRNA and two different proprietary LNPs (**B**). Genomic DNA was extracted from liver punches collected at the terminal endpoint and editing at the target locus was then assessed by NGS to determine the frequency of indels. Serum biomarker reduction was assessed by ELISAs specific to the species of interest. The percent change in biomarker levels were determined by comparing vehicle to treated animals at the terminal timepoint. All mice were C57Bl/6 background. Transgenic mice containing Target 1 or 2 were homozygous for the human gene-of-interest. Dotted line represents theoretical maximum hepatocyte editing based on the liver being composed of ~70% hepatocytes.[3]

Figure 3: *In vivo* gene editing proof-of-concept demonstrated in NHPs

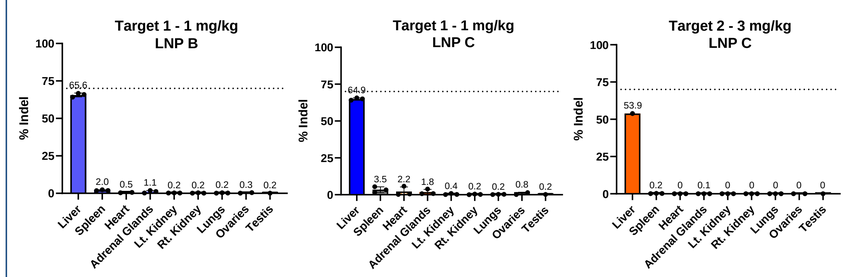
High *in vivo* liver editing and biomarker reduction observed in NHPs across various LNPs and gRNAs



NHPs were dosed intravenously with Vehicle or LNP at the indicated doses over a 1-hour period and monitored for 4 weeks. Genomic DNA was extracted from liver punches collected at the terminal endpoint and editing at the target locus was then assessed by NGS to determine the frequency of indels **(A,C)**. Target or disease-associated protein biomarker reduction in serum was assessed by ELISA **(A)** or other methodologies **(B,C)**. The percent change in biomarker levels was determined by comparing pre-dose samples to those collected each week. Target 1 **(A)** baseline within serum was determined by taking the average of the screening and pre-dose samples. The maximum level of biomarker reduction over the 4-week study is shown. Similar reduction in mRNA levels of each of the targets was also observed (data not shown). Dotted line represents theoretical maximum hepatocyte editing based on the liver being composed of ~70% hepatocytes.[3]

Figure 4: Minimal editing observed in non-target tissues

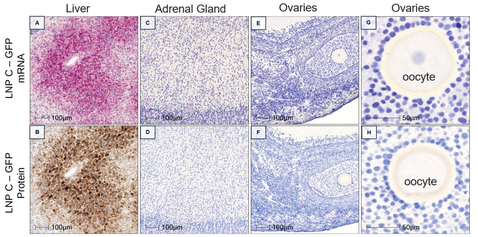
Minimal editing observed in non-target tissues at LNP doses that maximize on-target editing in the liver



Tissue punches were collected from NHPs at the study's terminal timepoint of 4 weeks. Genomic DNA was extracted from tissues and editing at the target locus was then assessed by NGS to determine the frequency of indels. Compared with the liver, non-target tissues, including gonads, showed negligible levels of editing. Dotted line represents theoretical maximum hepatocyte editing based on the liver being composed of ~70% hepatocytes.[3]

Figure 5: Favorable LNP on-target biodistribution profile

***In vivo* NHP results show favorable LNP on-target biodistribution profile**



At 24 hours after IV infusion of 1.5 mg/kg GFP mRNA cargo encapsulated in LNP B or LNP C, tissues were collected and fixed in 10% neutral buffered formalin (NBF). Routine histology processing and serial sectioning occurred prior to staining for GFP mRNA by in situ hybridization (ISH) via RNAscope and for GFP protein by immunohistochemistry (IHC). Slides were evaluated using a Zeiss Axioscan and representative images from one LNP group are shown. All GFP staining was compared to positive and negative controls.

GFP mRNA cargo is shown with red puncta in liver hepatocytes **(A)**. Corresponding translated protein **(B)** shows the same pattern in brown chromogen. Adrenal cortical cells show minimal signal **(C,D)**. Importantly, there is no positivity for either mRNA or protein in oocytes of the ovaries **(E, F, G, H)**. The IHC and ISH data shown is representative of LNP B and C.

Conclusions

- These studies represent the first successful demonstration of *in vivo* gene editing for liver targets using systemically delivered AsCas12a via LNPs in mouse and NHP models.
- Maximal gene editing levels were achieved within liver samples at different LNP doses, accompanied by significant reductions in disease-associated protein biomarkers in serum.
- Biodistribution studies confirmed high liver delivery with minimal delivery observed in non-target tissues, including no detectable signal in gonads. These data correlated with editing results.
- Translatability of this approach was validated across PHHs, transgenic mice, and NHPs with high levels of gene editing and disease-associated biomarker reduction observed across models.
- Our proprietary gene editing platform enables a 'plug-n-play' approach for rapid identification of effective LNP:cargo combinations, facilitating preclinical pipeline expansion and development.
- Continued optimization of LNP formulations, gRNA selection, and Cas mRNA will be critical for achieving clinical translation and addressing broader therapeutic targets.
- These studies establish a foundation for advancing novel genetic medicines targeting serious genetic diseases through efficient and specific delivery and gene editing technologies.

References

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