# Using Base Editing and LNP Delivery to Correct Disease-Causing Mutations Underlying Genetic Liver Diseases

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## Introduction

Base editing enables programmable single-base mutations in genomic DNA and has the potential to permanently cure serious genetic diseases. Realizing this potential requires development of safe and effective methods for delivery of base editing reagents to the intracellular compartments of target organs. LNPs are a clinically validated technology for delivery of RNA therapeutics. In this work, we have optimized LNPs for the delivery of mRNA encoding a base editor and guide RNA to hepatocytes. This optimization was conducted using a surrogate payload, a published adenine base editor (ABE) and a guide RNA that is conserved between rodents and non-human primates (NHP). In a parallel effort, we have developed disease-specific base editors and guide RNAs (gRNA) that can correct pathogenic mutations. When these therapeutic payloads are formulated in LNPs they are capable of efficiently correcting disease-causing mutations in the livers of transgenic mouse models.

## Genome Editing With Adenine Base Editors

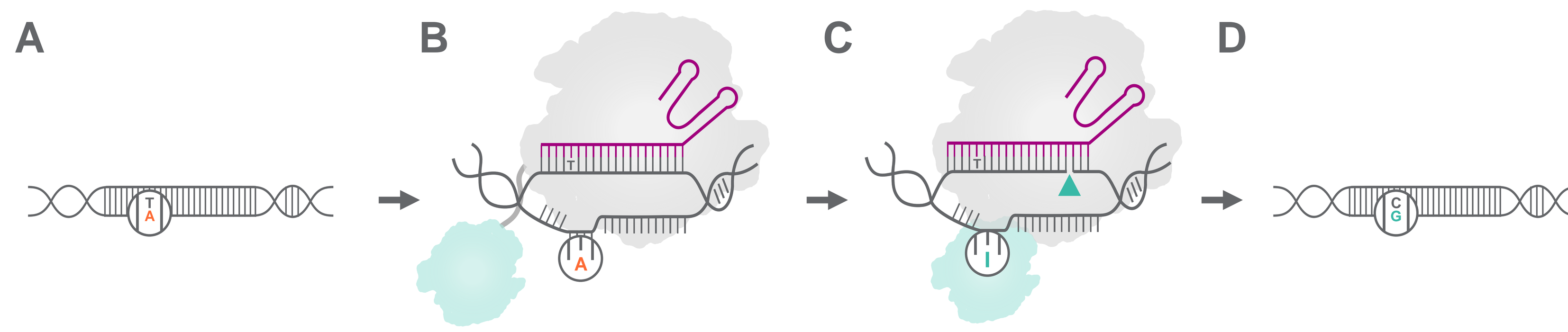


**Figure 1.** (A) Starting DNA sequence with the target base pair (A:T). (B) The adenine base editor (ABE) is a fusion protein consisting of an evolved TadA* deaminase (teal) connected to CRISPR-Cas enzyme (grey)[1,2]. The base editor binds to a target sequence that is complementary to the guide-RNA (magenta) and exposes a stretch of single-stranded DNA. (C) The deaminase converts the target adenine into inosine (which is read as guanine by DNA polymerases) and the Cas enzyme nicks (▲) the opposite strand. (D) The nicked strand is repaired completing the conversion of an A:T to G:C base pair.

## Optimizing LNP And Disease-specific Payload In Parallel

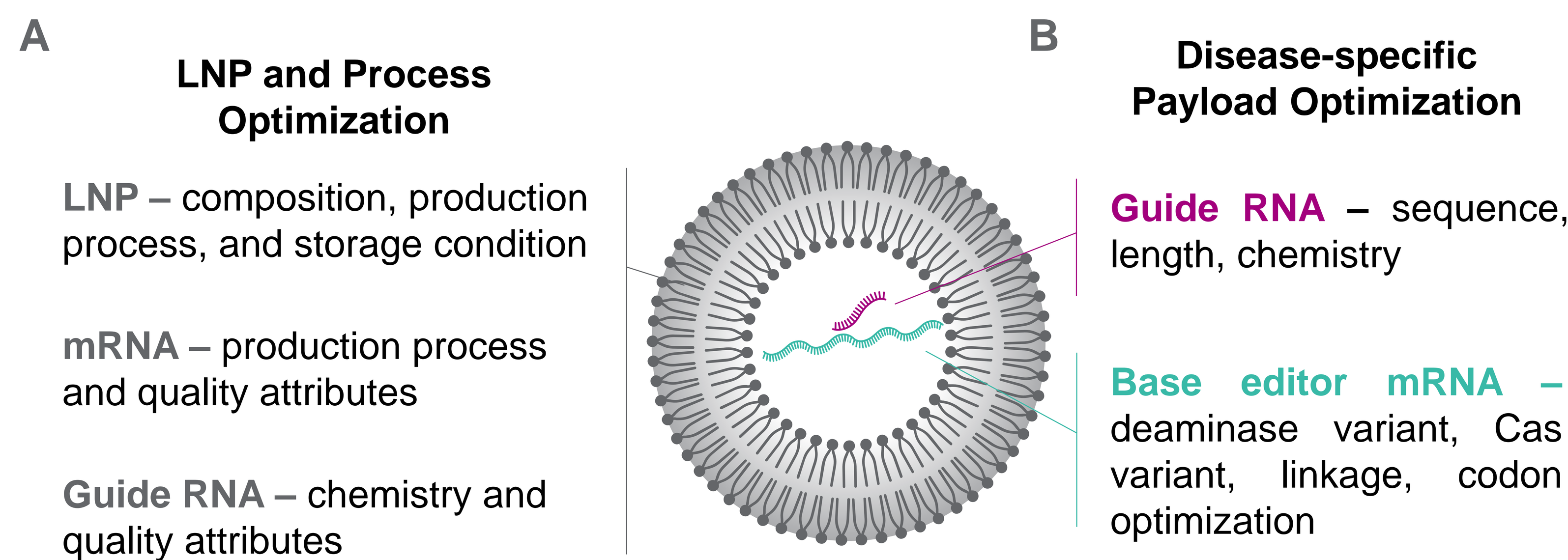


**Figure 2.** (A) Many features of an LNP formulation and the production processes of mRNA and guide RNA can be optimized irrespective of the sequence of the nucleic acid cargo. In this work, we selected a surrogate cargo which is the combination of an mRNA encoding an adenine base editor (ABE8.8)[2] and a guide RNA targeting a sequence within the ALAS1 gene that is conserved between rodent and NHP. These RNAs are expected to be similar in size to those used in disease-specific applications. (B) Disease-specific base editor and guide RNA are optimized in biologically relevant models. For these experiments, merely a prototype LNP formulation can be used to enable in vivo editing and the study of resulting pharmacological effects.

## Optimizing LNPs Encapsulating Surrogate Payload For In Vivo Base Editing

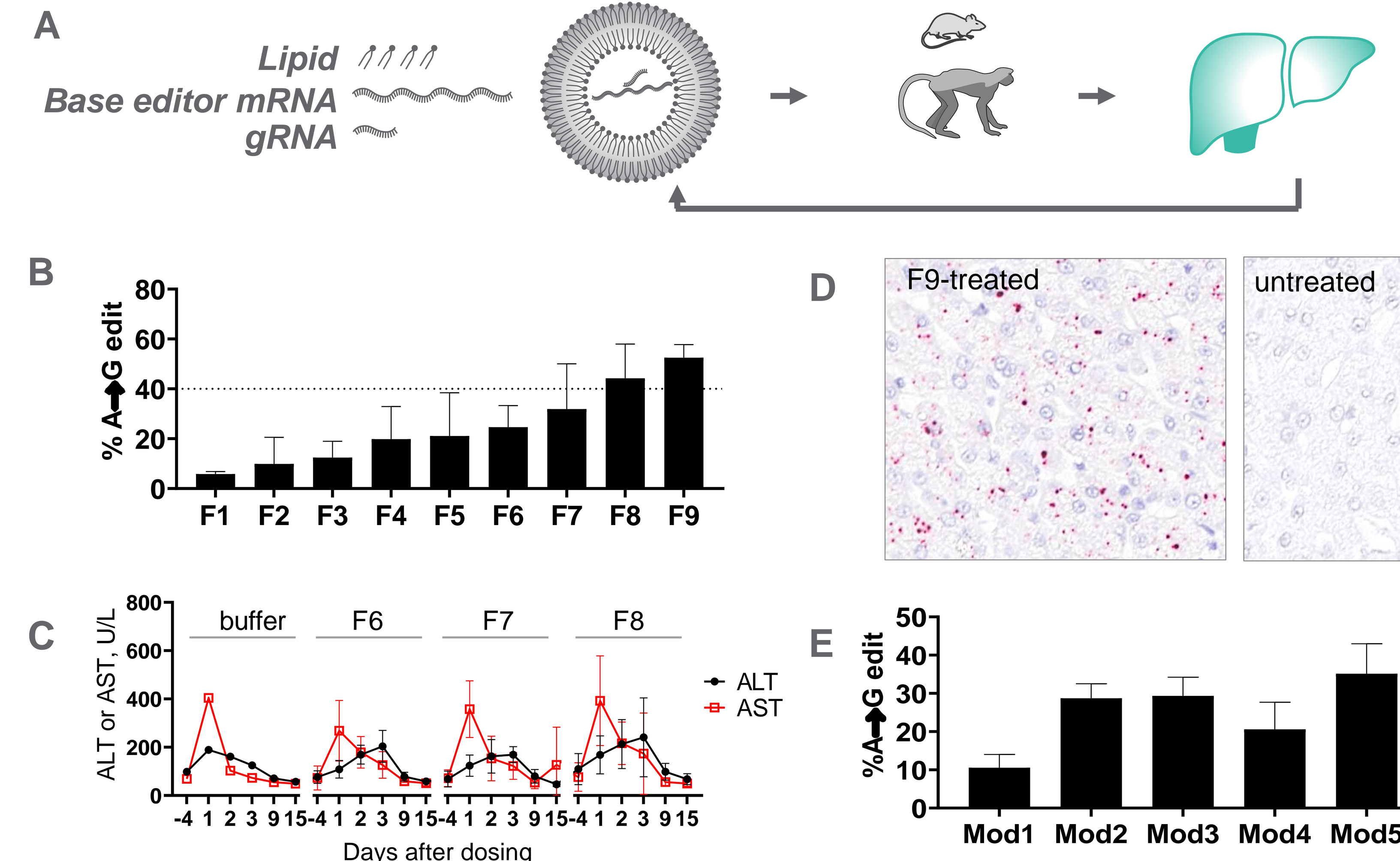


**Figure 3.** (A) Schematic depicting in vivo workflow for LNP optimization. A mRNA encoding ABE8.8 and gRNA were co-encapsulated in LNPs. (B) Editing efficiency in total liver extracts of NHPs improved from 6% to 52% at 1.5mg/kg total RNA by iterating through changes to the LNP formulation and mRNA production process. Data were pooled from multiple experiments (n=3 or 4). F6, F7, and F8 are a side-by-side comparison of three LNPs where the ionizable lipid and mRNA were kept constant (n=3). (C) Elevation of serum ALT and AST levels in treated NHPs was mild and transient, indicating good tolerability of the LNP formulations. (D) BaseScope in situ hybridization assay on fixed liver sections from F9-treated (left) vs untreated (right) NHPs using a probe specific against the mutant ALAS1 mRNA confirmed efficient editing of hepatocytes. (E) New gRNA modification strategies (chemically modified at 7-88% of the bases, Mod2-Mod5) were tested in mice yielding increased editing compared to control guide RNA (6% modified, Mod1). These modifications will be evaluated in NHPs.

## Preliminary Assessment Of LNP Formulation Stability

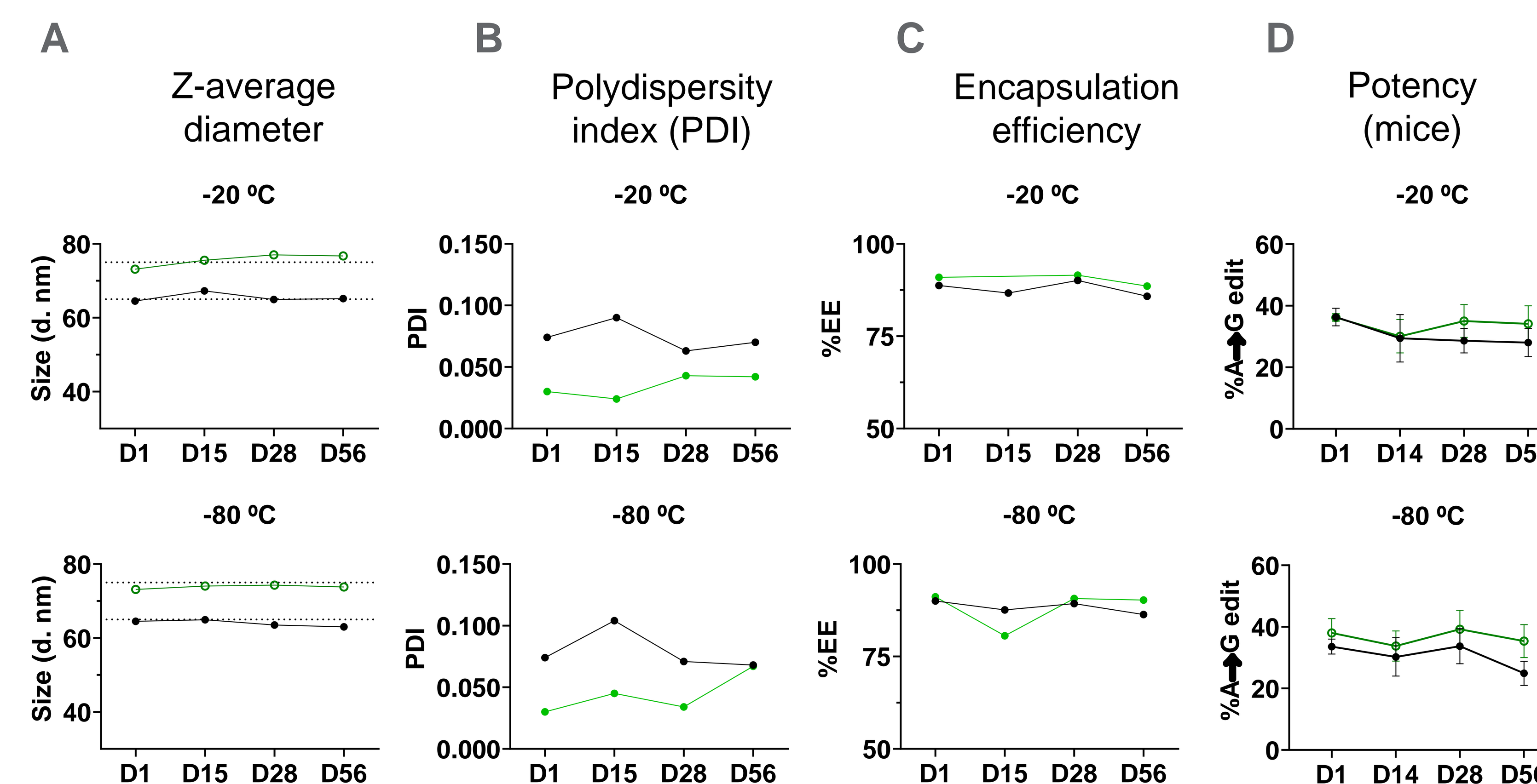


**Figure 4.** (A, B, C, D) Aliquots of LNP1 (Black solid dots) and LNP3 (Green empty circles) were stored at -20ºC and -80ºC and assayed for various biophysical characteristics as well as gene editing potency in mice. The formulations appear stable after storage of 8 weeks, the latest data point available.

## Optimizing Disease-specific Payload Using A Prototype LNP

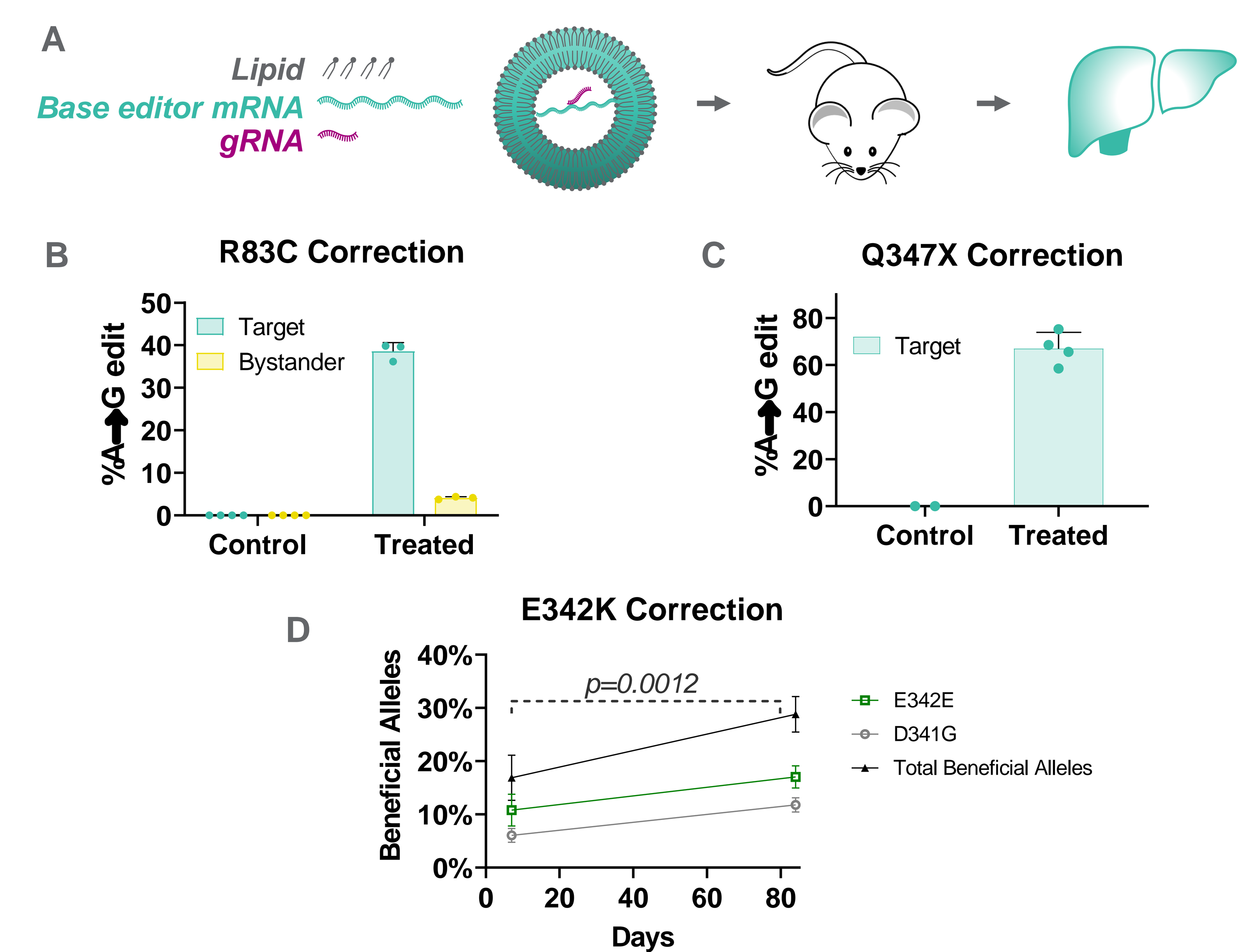


**Figure 5.** (A) Schematic depicting in vivo workflow. LNPs encapsulating disease-specific payload were dosed in transgenic mouse models of Glycogen-storage disease Type 1a or alpha-1 antitrypsin deficiency. (B, C) In transgenic mice heterozygous for mutant huG6PC, precise correction of R83C (B) and Q347X (C) is confirmed in 40% and 70% of total liver extracts, respectively. (D) In NSG-PiZ transgenic mice, precise correction of the E342K mutation yielded two beneficial alleles E342E and D341G. The beneficial alleles increased significantly at three months indicating that corrected hepatocytes may have a proliferative advantage

## Conclusions & Future Directions

Using an mRNA encoding ABE8.8 and gRNA targeting the ALAS1 gene as surrogate payload, we evaluated various LNP formulations and mRNA production processes to improve editing in the liver of NHPs from <10% to 52% at a total RNA dose of 1.5 mg/kg. The recent iterations demonstrated consistent 30-52% editing, were well tolerated at 1.5 mg/kg, and showed promising interim stability at -20ºC. New guide RNA modification strategies and continued optimization of LNP and mRNA is expected to further increase LNP potency and will be tested in NHPs in the future. In parallel, we have developed disease-specific base editors and guide RNAs that demonstrated promising editing in mouse models using prototype formulations. Future plans include merging these separate workstreams as we continue to optimize formulations on our path to develop important medicines for patients suffering from serious diseases.

## References And Disclosures

1. Gaudelli NM, Komor AC, Rees HA, et al. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature, 551:464–471 (2017)
2. Gaudelli, N.M., Lam, D.K., Rees, H.A. et al. Directed evolution of adenine base editors with increased activity and therapeutic application. Nat Biotechnol (2020).

This work was funded by Beam Therapeutics, a public company developing base edit technology for human therapeutics. Delai Chen, Shailendra Sane, Raymond Yang, Robert Dorkin, Eric Zhang, Dongyu Chen, Xiao Luo, Yvonne Aratyn-Schaus, Brian Cafferty, James Tam, Krishna Ramanan, Jeffrey Marshall, Aaron Larsen, Michael S Packer, Sarah Smith, Carlo Zambonelli, Francine M Gregoire, Giuseppe Ciaramella, Manmohan Singh are all employees of Beam Therapeutics.


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