# Rescue of AAT-Z mutant protein aggregation in PiZ mice after treatment with an LNP delivered RNA editing oligonucleotide targeting the E342K mutation.

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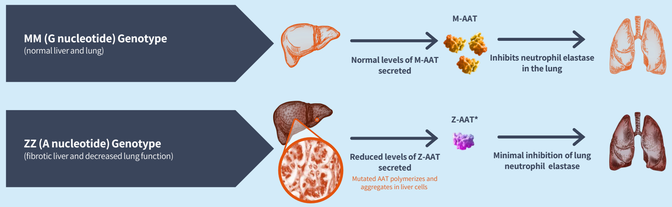
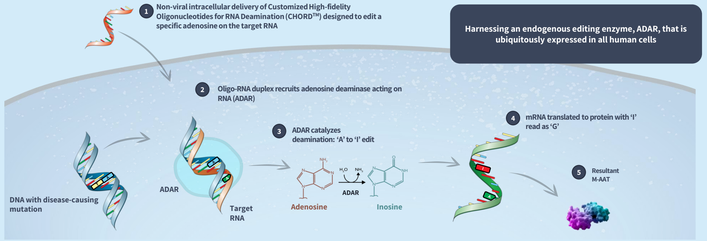
## Rationale

Alpha-1 antitrypsin (AAT) deficiency (AATD) is a rare genetic disorder that results in abnormal protein aggregate formation within hepatocytes. The most prevalent mutation in humans for AATD is the Glu342Lys (also known as the E342K or Z) point mutation. Korro is using an RNA editing approach to correct the E342K (G>A) mutation to target the Z allele. RNA editing is a natural physiological process that occurs in cells where a specific single base edit is mediated by an enzyme called Adenosine Deaminase Acting on RNA ("ADAR"). Korros proprietary RNA editing approach involves co-opting this endogenous editing system via an engineered oligonucleotide to introduce precise edits to RNA. To study the pharmacological activity of Korro Bio's LNP delivered oligonucleotide *in vitro* and *in vivo*, the PiZ transgenic mouse model of AATD liver disease was used. The livers of these mice have significant Z protein aggregation and secrete the mutant human Z-AAT protein into circulation. In vitro data demonstrated that an LNP delivered oligonucleotide can edit *in vitro* hepatocytes derived from PiZ mice. *In vivo,* sub chronic dosing resulted in editing of the E342K mutation that led to newly repaired M-AAT protein. The function of this protein was assessed in a neutrophil elastase (NE) inhibition assay and translated to functional AAT. Data demonstrated that elastase inhibition correlated with the percent editing observed in the livers of the mice. These data support utilizing RNA editing to correct the mutated protein to achieve functioning AAT protein levels in PiZ mice and the potential to translate to similar results in AATD patients.

## AATD caused by a single missense (G-to-A) mutation in the *SERPINA1* Gene in Liver




## RNA editing to transiently correct the AATD ZZ Genotype




## PiZ Transgenic Mouse model


## KB020794 editing in MZ PHH and PiZ PMH *in vitro* systems

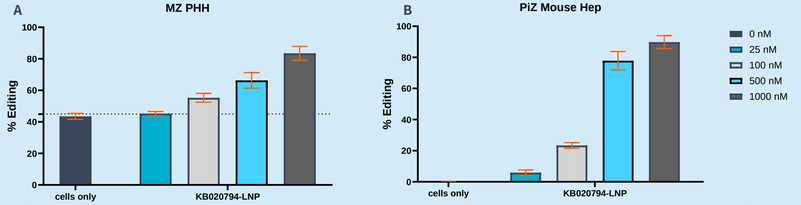



**Figure 1:** A) MZ donor derived primary human hepatocytes (PHH) treated with KB020794-LNP delivery demonstrate editing of the Z allele in a dose responsive manner approaching 90% at the top dose. Editing was measured by NGS at 48 hours post dose (n=4) B) Isolated PiZ PMH treated with KB020794-LNP demonstrate editing of the E342K allele in a dose responsive manner approaching 90% at the top dose. Editing was measured by NGS at 48 hours post dose (n=4). Demonstration of strong translation between PiZ mouse model and human cell system.

## KB020794 editing *in vivo* PiZ mouse model

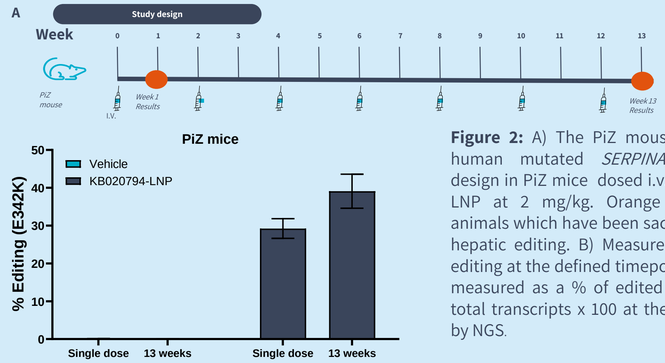



**Figure 2:** A) The PiZ mouse expresses the human mutated *SERPINA1* gene. Study design in PiZ mice dosed i.v. with KB020794-LNP at 2 mg/kg. Orange dots represent animals which have been sacrificed to look at hepatic editing. B) Measurement of hepatic editing at the defined timepoints. Editing was measured as a % of edited transcripts over total transcripts x 100 at the E342K RNA site by NGS.

## Secretion of M-AAT and neutrophil elastase inhibition

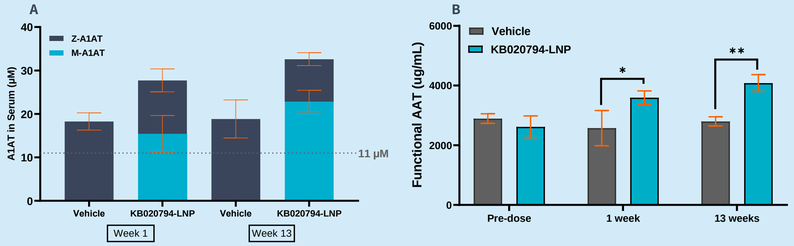



**Figure 3:** A) Measurement of serum AAT concentrations by absolute quantification using LC-MS/MS in the PiZ mouse model treated with KB020794-LNP. The total AAT represents a combination of M-AAT + Z-AAT in circulation. B) Measurement of human neutrophil elastase activity using the mouse serum from the PiZ mouse model treated with KB020794-LNP. One-way ANOVA *$P$<.05, **$P$<.01.

## Clearing of aggregation in PiZ mouse livers

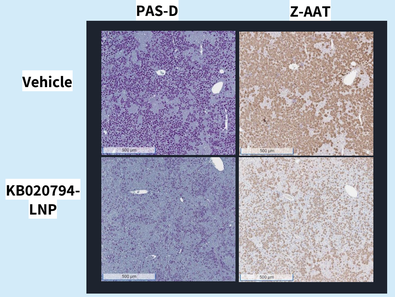



**Figure 4:** Representative Periodic Acid-Schiff-Diastase (PAS-D) (purple) stain and Z-AAT (brown) stain sections from PiZ mice dosed for 13 weeks. Vehicle treated vs KB020794-LNP, images captured at 10X magnification.

## Conclusion

- KB020794 is a novel oligonucleotide encapsulated in a lipid nanoparticle for liver-directed delivery. This is designed to bind to the Z allele mutation site of AATD patient RNA and recruit ADAR to edit the sequence from A to I, leading to repair of the mutated allele and translation of M-AAT. This approach will treat AATD by correcting the underlying genetic mutation causing the disease.
- *In vitro* dose response demonstrates good translation between MZ and PiZ hepatocytes when treated with KB020794-LNP.
- KB020794-LNP delivery in the PiZ mouse model results in approx. 40% editing and increased M-AAT levels (~20µM) in circulation to above a clinically relevant threshold.
- Newly made WT-protein demonstrated functional activity in the neutrophil elastase inhibition assay.
- Representative images stained for PAS-D and Z-AAT show a reduction in aggregation correlation to the levels in the serum indicating potential to correct and alleviate liver disease.

Disclosure of financial interests:
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