KRRO-110, an RNA Editing Oligonucleotide Encapsulated in a Lipid Nanoparticle (LNP) Delivered to Liver Cells for the Treatment of Alpha-1 Antitrypsin Deficiency (AATD)

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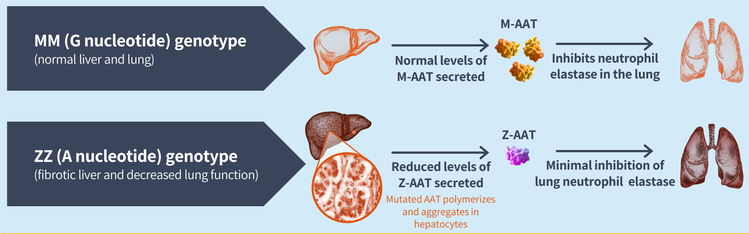
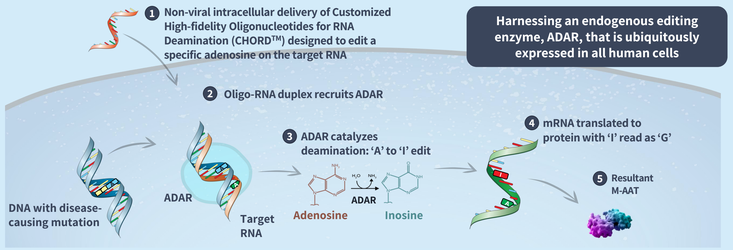
Rationale

AATD is an inherited, autosomal recessive genetic disorder that is often caused by single nucleotide variants (SNV) in the *SERPINA1* gene encoding for alpha-1 antitrypsin (AAT), for which the most common mutation is classified as the **E342K** mutation also known as the Z allele. The mutation is a G->A mutation which introduces an amino acid change from Glutamic Acid (E) coding for M-AAT to a Lysine (K) coding for Z-AAT. This mutation is a significant risk factor in the development of liver and/or progressive lung disease. RNA editing is a natural physiological process mediated by an enzyme called Adenosine Deaminase Acting on RNA (ADAR) that occurs in cells where an Adenosine (A) on RNA is edited to an Inosine (I) that is translated as a Guanosine (G). Korro Bio's RNA editing approach involves co-opting this endogenous RNA editing system via KRRO-110 which is a proprietary engineered oligonucleotide encapsulated in a LNP to introduce precise and transient edits to the *SERPINA1* RNA in the liver.

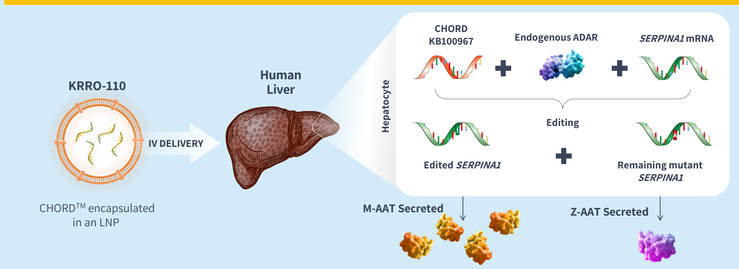
AATD caused by a single missense (G-to-A) mutation in *SERPINA1* gene in liver



RNA editing to transiently correct the AATD ZZ genotype



KRRO-110 designed to correct pathogenic Z-AAT protein to M-AAT



RNA editing in human *in vitro* systems

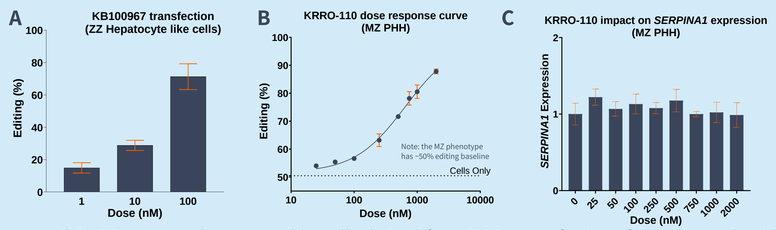


Figure 1: A) In ZZ genotype hepatocyte like cells derived from iPSCs, transfection of the oligonucleotide component KB100967 leads to dose responsive RNA editing, exceeding 70% at 100 nM, measured by next generation sequencing (NGS) at 48 hours post dose (n=3) B) MZ donor derived primary human hepatocytes (PHH) treated with KRRO-110 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) C) KRRO-110 delivery in MZ PHH does not change *SERPINA1* expression levels at increasing doses. Expression was analyzed via digital PCR (dPCR) using primers and probes designed to the *SERPINA1* transcript. Results are not statistically significant for treated groups vs 0 nM control (One-way ANOVA).

KB100967 is specific to the human *SERPINA1* gene

Table 1: No predicted off-target hits based on KB100967 sequence	
Number of mutations	Number of predicted off target hits
0	0
4	0
8	0
12	0

Table 1: To characterize the specificity of KB100967, we used *in silico* approaches based on sequence homology across the human transcriptome and found no off-target hits even when allowing for 12 mutations on the sequence.

KRRO-110 has negligible cis off-target editing on the *SERPINA1* gene

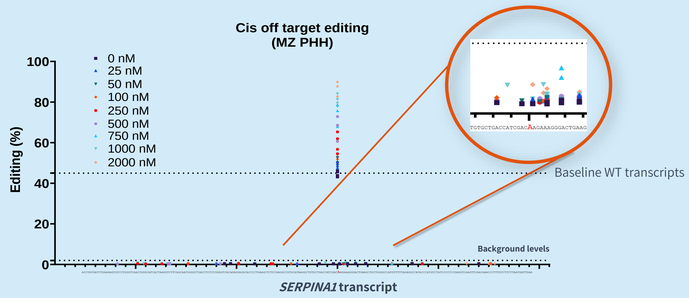


Figure 2: *In vitro* analysis of off-target editing at all "A" nucleotides 100 bases upstream and downstream of the target site (E342K) in MZ PHH. Based on NGS, all sites other than the E342K target resulted in less than 1% RNA editing, with similar editing between treated cells and negative controls.

KRRO-110 does not impact editing of natural ADAR targets

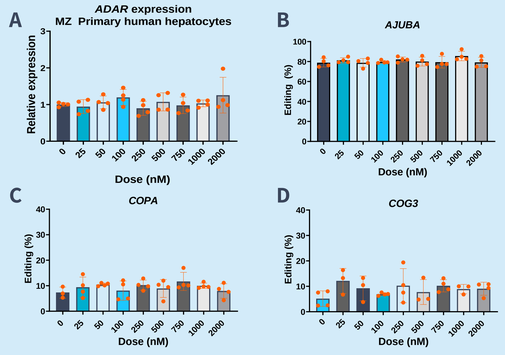


Figure 3: A) *ADAR* gene expression (encodes ADAR1 protein) expression by qRT-PCR and B-D) editing at known endogenous editing sites *AJUBA*, *COPA*, and *COG3* which showed no change with KRRO-110 treatment, reflecting minimal disruption to ADAR's ability to edit these sites. Note: *ADARB1* expression (encodes ADAR2 protein) was minimally expressed in MZ PHH. Results are not statistically significant for treated groups vs 0 nM control (One-way ANOVA).

KRRO-110 RNA editing *in vivo* NSG-PiZ mouse model

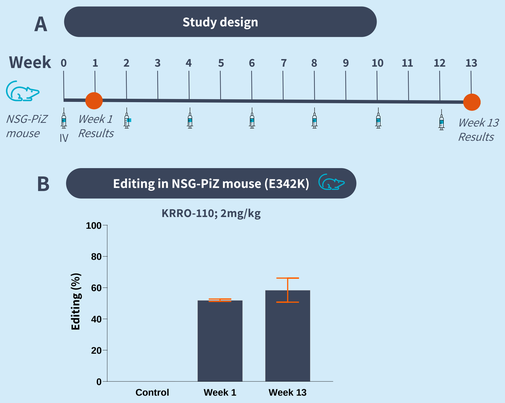


Figure 4: A) The NSG-PiZ mouse expresses the human mutated (E432K) *SERPINA1* gene. Study design in NSG-PiZ mice dosed i.v. with KRRO-110 Q2W at 2 mg/kg. Orange dots represent animals which have been sacrificed to look at hepatic editing. B) Measurement of hepatic editing at the defined timepoints. Editing was measured as a % of edited transcripts over total transcripts x 100 at the E342K RNA site by NGS.

Secretion of M-AAT and inhibition of human neutrophil elastase

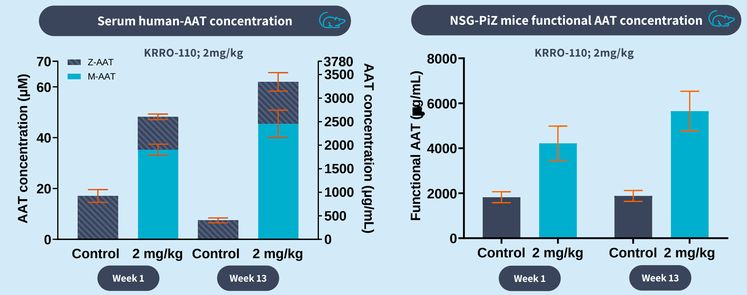


Figure 5: A) Measurement of serum human AAT concentrations by absolute quantification using LC-MS/MS in the NSG-PiZ mouse model treated with KRRO-110. The total AAT represents a combination of M-AAT + Z-AAT in circulation. B) Measurement of functional AAT as human neutrophil elastase inhibition using the mouse serum from the NSG-PiZ mouse model treated with KRRO-110.

Editing at surrogate target site in mouse model translated to higher species

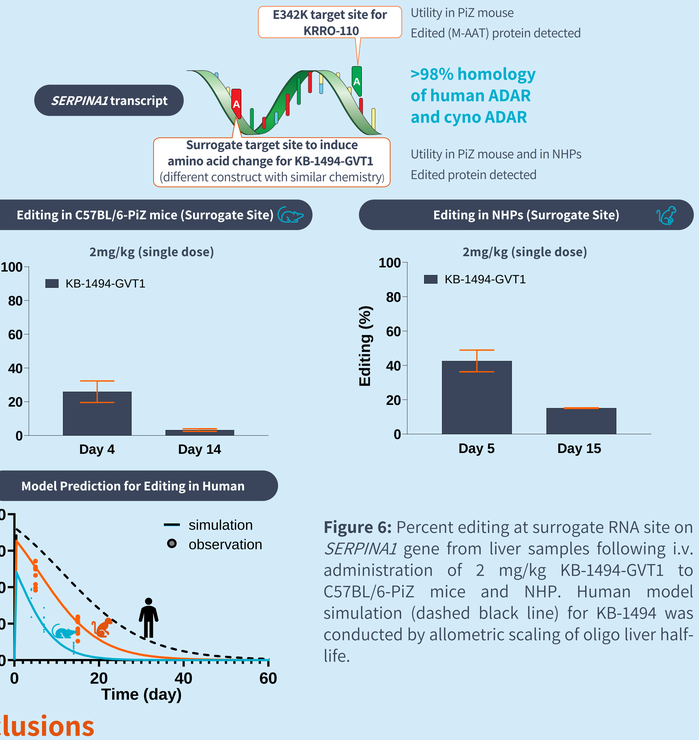


Figure 6: Percent editing at surrogate RNA site on *SERPINA1* gene from liver samples following i.v. administration of 2 mg/kg KB-1494-GVT1 to C57BL/6-PiZ mice and NHP. Human model simulation (dashed black line) for KB-1494 was conducted by allometric scaling of oligo liver half-life.

Conclusions

- KRRO-110 is a novel oligonucleotide encapsulated in a LNP for liver-directed delivery. This therapeutic 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.
- Treatment of human hepatocytes with Z mutation demonstrates dose responsive editing with endogenous human ADAR and limited impact on target transcript, cis off target editing, or overall ADAR function.
- KRRO-110 delivery in the PiZ mouse model results in >50% editing and increased M-AAT levels (~40μM) in circulation to above a clinically relevant threshold and showed neutrophil elastase inhibition.
- Editing of an endogenous *SERPINA1* site in NHPs leads to secretion of edited protein and supports translation to larger species.

Next Steps

- Regulatory filing for First-in-Human study of KRRO-110 in AATD patients is anticipated in the second half of 2024

Disclosure of financial interests:
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