Use of Adenine Base Editors to Precisely Correct the Disease-Causing PiZ Mutation in Alpha-1 Antitrypsin Deficiency

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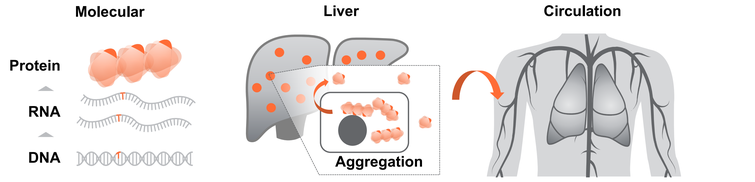
Liver and Lung Manifestations of Alpha-1 Antitrypsin Deficiency



- Caused by mutation within *SERPINA1* gene
- Often the severe PiZ mutation (E342K)
- Protein aggregation can cause fibrosis and/or cirrhosis
- No approved treatment other than transplant
- Deficient anti-elastase function leads to lung damage and emphysema
- *Augmentation* therapy slows progression
- Some patients require lung transplant

Could correction of the PiZ mutation via lipid-nanoparticle-mediated base editing in the liver resolve both liver and lung pathologies?

Optimizing an Adenine Base Editor to Correct the PiZ Mutation

B

Editor ID	TadA Architecture	TadA* variant	PAM variant
ABEvar1	Dual	7.10	1
ABEvar2	Dual	7.10	2
ABEvar3	Dual	7.10	3
ABEvar4	Dual	A	3
ABEvar5	Single	A	3
ABEvar6	Single	B	3
ABEvar7	Single	C	3
ABEvar8	Single	D	3
ABEvar9	Single	E	3
ABEvar10	Single	E	4
ABEvar11	Single	E	5
ABEvar12	Single	F	5

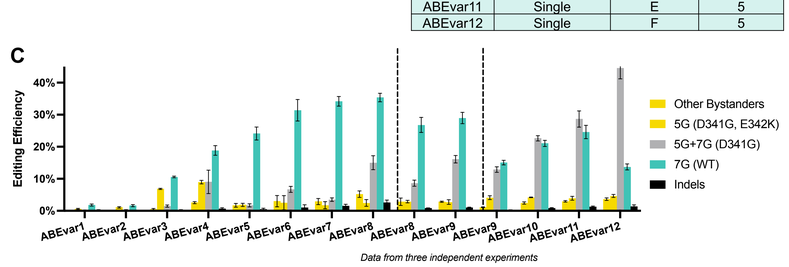


Figure 1 (A) Target DNA sequence and amino acid translation for the E342K (PiZ) mutation. (B) Description of base editor constructs interrogating various spCas9 NGC PAM variants and TadA* mutants.[1] (C) Allele frequencies assessed by high-throughput targeted amplicon sequencing in primary PiZZ fibroblasts (GM11423, Coriell) transfected with base editor mRNA and gRNA. In addition to precise correction (7G), a significant amount of linked bystander editing (5G+7G) was also observed.

The 5G+7G Allele Yields a 'Corrected' D341G A1AT Protein

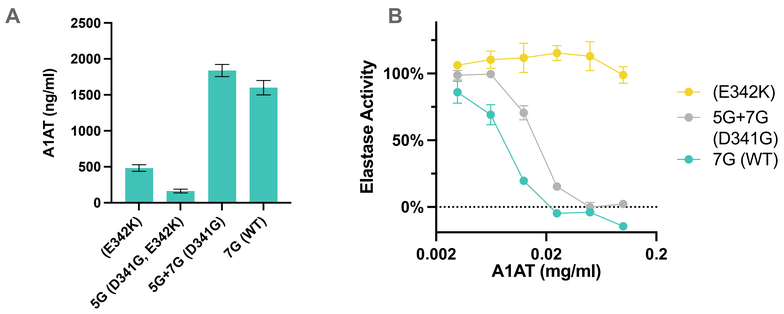


Figure 2 (A) Alpha-1 Antitrypsin (AAT) secretion assay in HEK293T transfected with expression vectors. (B) Elastase inhibition assay with purified AAT proteins. D341G AAT exhibits wild-type levels of secretion and anti-elastase activity.[2]

Optimizing In Vivo Correction of the PiZ mutation in NSG-PiZ Mice

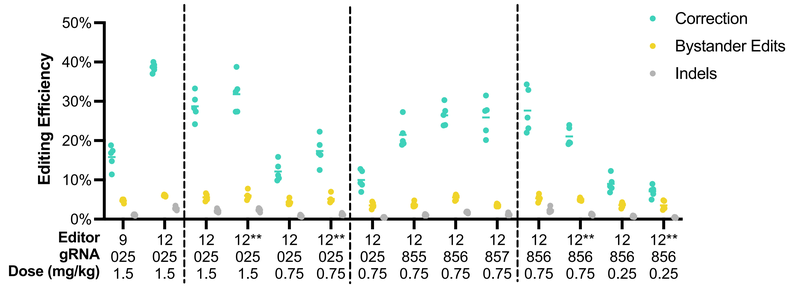


Figure 3 Base editing efficiencies in total liver extracts from 5–7-week-old NSG-PiZ transgenic mice (Jackson Laboratory) assessed 1 week after dosing via IV injection with lipid nanoparticles (LNP) carrying base editor mRNA and gRNA. Editor 12** possesses the same amino acid sequence as editor 12 but is expressed from a codon optimized mRNA. gRNA855-857 are the same nucleotide sequence as gRNA025 but contain different chemical modifications. Depicted data is from four separate studies.

In Vivo Correction Increases Functional Human A1AT in Serum

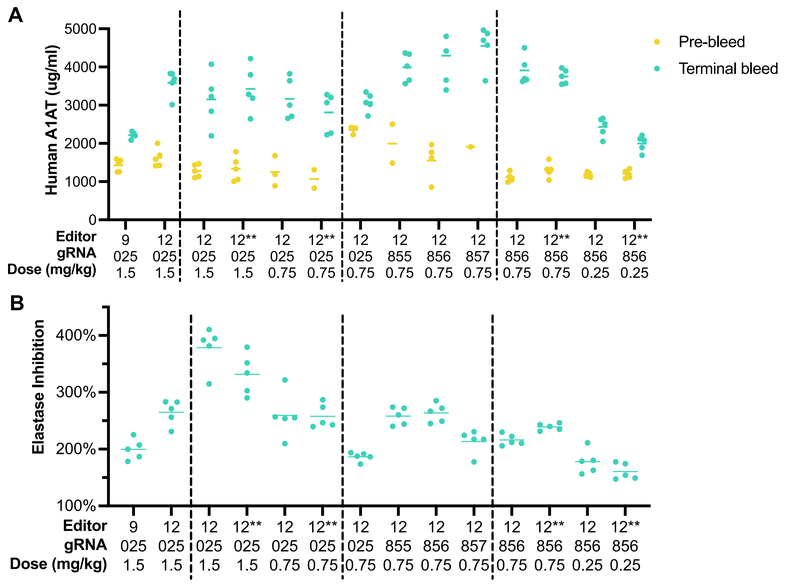


Figure 4 (A) Serum human AAT measured by immunoassay (Meso Scale Discovery). (B) Human neutrophil elastase inhibition capacity of terminal serum samples (expressed as a percentage of inhibitory activity of pre-bleed). Depicted data is from four separate studies.

In Vivo Correction Reduces PiZ Globule Burden in Mouse Liver

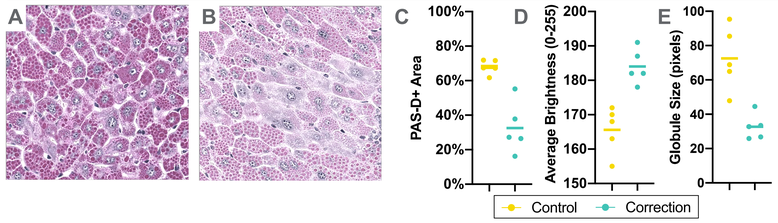


Figure 5. Representative Periodic Acid-Schiff-Diastase (PAS-D) stained sections of mouse livers from control group (A) and correction group (B). Images at 40x magnification were subject to color thresholding (ImageJ) to calculate (C) percent PAS-D stained area and (D) average pixel brightness in PAS-D stained regions.

Durable Editing and Increased Serum AAT in Adult NSG-PiZ Mice

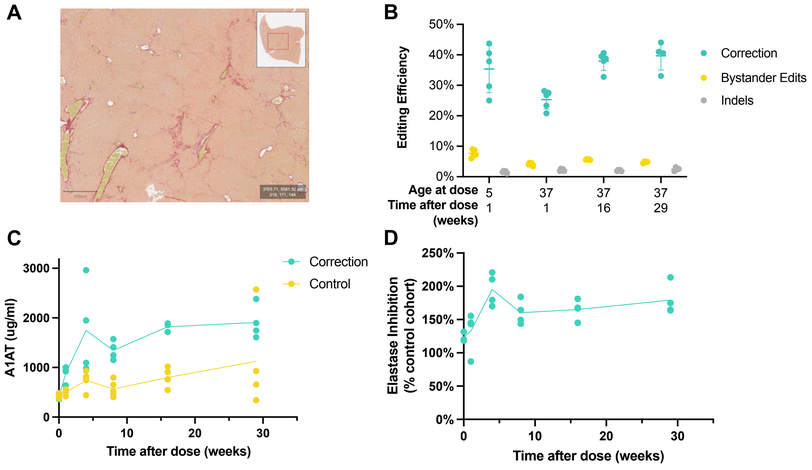


Figure 6 Comparison of correction LNP formulated with variant 12 editor mRNA and sgRNA025 in subjects dosed at 5 and 37 weeks of age relative to a control LNP formulated with BE4 mRNA and a gRNA targeting PCSK9.[3] (A) Representative Picrosirius-red-stained liver section demonstrating modest fibrosis around the time of treatment (samples collected from subjects treated with control LNP at 37 weeks of age and collected 1 week after treatment). (B) Base editing efficiency in total liver extracts. Results indicate comparable base editing in 37-week-old subjects relative to 5-week-old subjects and a modest increase in base editing efficiency over time due to proliferative advantage of corrected hepatocytes. (C) Serum human AAT measured by immunoassay (Meso Scale Discovery). (B) Human neutrophil elastase inhibition capacity of serum samples relative to age matched control cohort.

Conclusions

In these studies, base editing reagents were engineered to correct the disease-causing PiZ mutation in *SERPINA1*. Although precise correction (7G) was among the most common editing outcomes in transfected cell cultures, an abundant allele (5G+7G) was found to yield a 'corrected' D341G AAT protein possessing functional properties comparable to wild-type.

Using LNP-delivery technology, this base editing approach was assessed in the NSG-PiZ transgenic mouse model. LNPs formulated with end-modified gRNA025 and mRNA encoding the latest variant 12 editor yielded up to 40% and 17% editing when dosed at 1.5 mg/kg and 0.75 mg/kg, respectively. Chemically-modified gRNAs yielded more potent base editing, ~27% and ~9% correction at 0.75 mg/kg and 0.25mg/kg, respectively. A physiologically relevant ~2-fold increase in serum human A1AT was observed after a 0.25 mg/kg dose of LNP formulated with var12/sgRNA856.

This base editing approach was also evaluated in older PiZ mice. LNP-mediated base editing was efficient and durable despite subject age and early liver fibrosis. This treatment context is likely more comparable to what could be encountered clinically in Alpha-1 patients.

Future Directions

We have initiated studies to characterize the potential for off-target genome modification with these reagents. Further development of this research program may require additional refinements to the mRNA, gRNA, and LNP formulation.

References and Disclosures

1. Werder RB, Kaserman JE, Packer MS, Lindstrom-Vautrin J, Villacorta-Martin C, Young LE, Aratyn-Schaus Y, Gregoire F, Wilson AA. Adenine base editing reduces misfolded protein accumulation and toxicity in alpha-1 antitrypsin deficient patient iPSC-hepatocytes. Mol Ther. 29(11):3219-3229 (2021).
2. Packer MS, Chowdhary V, Lung G, Cheng LI, Aratyn-Schaus Y, Leboeuf D, Smith S, Shah A, Chen D, Zieger M, Cafferty BJ, Yan B, Ciaramella G, Gregoire FM, Mueller C. Evaluation of cytosine base editing and adenine base editing as a potential treatment for alpha-1 antitrypsin deficiency. Mol Ther. 30(4):1396-1406 (2022).
3. Chadwick AC, Wang X, Musunuru K. In Vivo Base Editing of PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) as a Therapeutic Alternative to Genome Editing. Arterioscler. Thromb. Vasc. Biol., 37:1741-1747 (2017).

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