Amelioration of Alpha-1 Antitrypsin Deficiency Diseases with Genome Editing in Transgenic Mice

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ASGCT 2017
Disclosures

- Dr. Jeffrey H. Teckman is a consultant to Editas Medicine. Keith Blomenkamp is employed by Saint Louis School of Medicine.
Alpha-1 Antitrypsin Deficiency

AAT Protein → Misfolding → Polymerization

Liver Globules by Periodic Acid-Schiff Staining

Fibrosis, Cirrhosis, Hepatocellular Carcinoma

Z(E342K)

Alveolar Damage

Emphysema, COPD

Fairbanks, KD, American Journal of Gastroenterology (2008); Lomas, DA, Clin Med (2005); PDB ID: 3NE4
Transgenic mice harbor the intact human SERPINA1-Z locus (PiZ)
- Positive staining of hAAT-Z globules with Periodic Acid Schiff + Diastase (PAS-D)
- Mouse SerpinA1 loci are still present

![Diagram of Human SERPINA1 locus with PiZ mutation](image)

**PAS-D Staining**

- **PiZ Mouse**
- **Wildtype**
hSERPINA1 Gene Editing to Treat AATD Diseases

Human SERPINA1

5’UTR

IA  IB  IC  II  III  IV  V  3’UTR

Z Mutation: E342K

NHEJ Strategy

Non-Homologous End Joining
Nonsense-mediated Decay

HDR Strategy

Homology-Directed DNA Repair

Reduce AAT-Z Globules
Alleviate Liver Burden
Improve Lung Protection
Gene Editing of Exon II Decreases hSERPINA1 Expression

NGS

RT-qPCR

Percent Editing

hSERPINA1 Expression relative to B2m by RT-qPCR

PBS  AAV8-CRISPR

PBS  AAV8-CRISPR

34%

4%
Gene Editing of Exon II Reduces Circulating AAT-Z

ELISA of Human AAT in Mouse Serum

Serum hAAT (µg/ml)

Days post injection

PBS
AAV8-CRISPR

22%
1%

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Gene Editing of Exon II Reduces AAT-Z Globules in Liver

PAS-D Staining of Livers on Day 35

**PBS**

**AAV8-CRISPR**

Quantitation of PAS-D staining

![Graph showing quantification of PAS-D staining](image)
Dual-Vector HDR Approach to Correct the Z Mutation

Human SERPINA1
5'UTR

IA  IB  IC

Z Mutation: E342K
3'UTR

Donor
1.28e14 vg/kg

AAV8

Cas9
5.0e13 vg/kg

hU6
sgRNA

Homology Arm

Unedited
Insertions
Deletions
Correction by HDR

Outcomes on Exon V
Efficient Reduction of hSERPINA1 Expression in vivo

RNAseq of Total RNA

- hSERPINA1 expression relative to B2M
- PBS: 14%
- AAV8-Cas9 + AAV8-Donor: 3%

ELISA of Human AAT in Mouse Serum

- PBS: 1%
- AAV8-Cas9 + AAV8-Donor: 3%

Days post injection

Serum AAT (μg/ml)
Wild-Type hAAT Expression Restored in PiZ Livers

RNAseq of Total Liver RNA

Percent Correction of the Z Mutation

- PBS
- AAV8-Cas9 + AAV8-Donor

5%
Summary

- NHEJ approach disrupts hSERPINA1 loci in PiZ transgenic mice, dramatically reducing AAT-Z in circulation and AAT-Z aggregation in hepatocytes.

- HDR approach corrects the Z mutation in hSERPINA1 in vivo resulting in reduction of circulating AAT-Z and restoration of wild-type PiM expression.

- Due to limitations in current models, a novel PiZ transgenic mouse would be required to assess the potential impact of gene correction on lung disease caused by PiZ mutations.

- CRISRP/Cas9, in combination with AAV delivery systems, has the potential to be developed as a therapy for AATD patients with the PiZZ genotype.
Acknowledgements

Charlie Albright  
Vic Myer  
Andrew Hack  
Haiyan Jiang  
Michael Stefanidakis  
Georgia Giannoukos  
Dawn Ciulla

Viral Vector Core  
Guangping Gao  
Qin Su  
Ran He  
Jun Xie