Reduction of hepatic Z-alpha1 antitrypsin by RNA interference prevents and reverses liver disease including hepatic mitochondrial injury in the PiZ mouse model

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INTRODUCTION

Autosomal co-dominant genetic disorder alpha-1 antitrypsin deficiency (AATD) causes pulmonary and liver disease. Individuals homozygous for the mutant Z allele accumulate polymers of Z-AAT protein in hepatocytes, where AAT is primarily produced, resulting in hepatocyte injury and fibrosis that may over time lead to cirrhosis and hepatocellular carcinoma. The accumulated polymers cause oxidative stress, mitochondrial damage, and increased autophagic activity and apoptosis. Injury to mitochondria, dilated endoplasmic reticulum (ER), microvesicular fat and other ultrastructural changes can be visualized by electron microscopy (EM).

AIM

Adult PiZ mice were treated with RNAi-based therapeutic ARC-AAT to reverse the AATD-associated liver disease phenotype.

METHODS

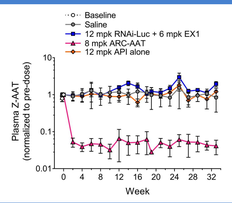
ARC-AAT, consisting of RNAi trigger (the RNAi-AAT API) plus an endosome-release agent (EX1), was evaluated in the PiZ mouse model that harbors the human Z-AAT gene and recapitulates AATD liver disease. Male PiZ mice were 11-17 weeks old at baseline. They were treated for 32-33 weeks with biweekly (Q2W) intravenous injections of 8 mg/kg (mpk) ARC-AAT (8 mpk RNAi-AAT API + 4 mpk EX1) or with saline as a control. Additional control groups were treated with 12 mpk RNAi-AAT API alone or 12 mpk control RNAi trigger (RNAi-Luc) delivered with 6 mpk EX1.

- Plasma Z-AAT was measured by ELISA (Abcam).
- Soluble (monomeric) and insoluble (polymeric) Z-AAT in the liver were measured by semi-quantitative Western blotting.
- Globules of Z-AAT polymer aggregates were measured by PAS-D staining and ImageJ quantitation, 3 fields of view per liver specimen.
- Liver inflammation was assessed by histological evaluation with H&E staining and morphometric analysis.
- Expression of genes previously implicated in liver injury and development of fibrosis was measured by RT-qPCR.
- The intracellular state of hepatocytes, including dilated ER, mitochondrial injury, and autophagosomes were assessed by EM.

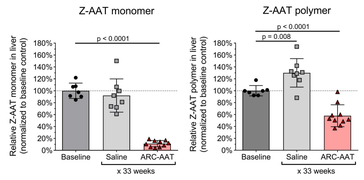
RESULTS

Plasma Z-AAT measurement

- 95-98% plasma Z-AAT reduction relative to baseline for duration of Q2W ARC-AAT treatment.
- No reduction of Z-AAT from the API alone nor from control RNAi trigger (RNAi-Luc).

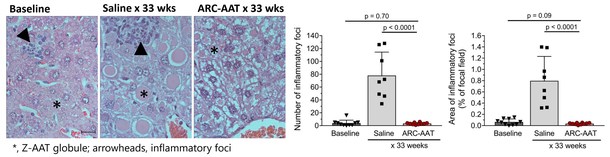


Soluble (monomeric) and insoluble (polymeric) Z-AAT protein in liver



- Monomeric Z-AAT protein reduced 89%.
- Polymeric Z-AAT reduced 42%, relative to baseline.
- Polymeric Z-AAT in age-matched saline control group increased 30%.

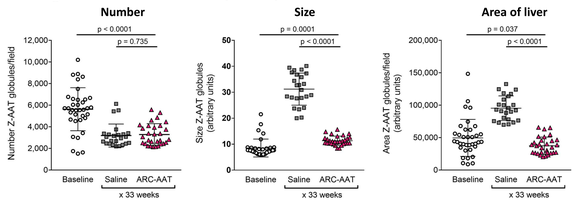
Histological evaluation of globules and inflammation (H&E)



*, Z-AAT globule; arrowheads, inflammatory foci

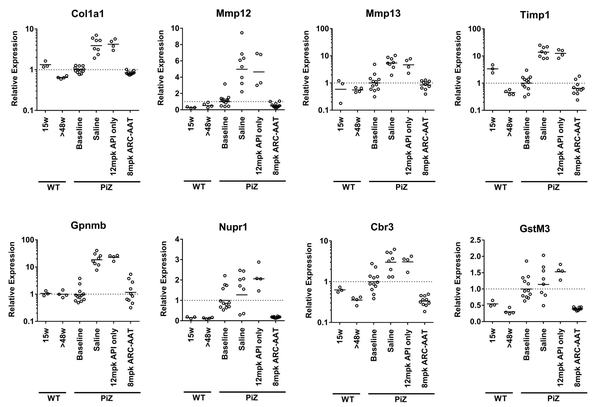
- Baseline: numerous globules but minimal inflammatory infiltration
- Saline x 33 weeks: very large globules and 20-fold more inflammatory foci than at baseline
- ARC-AAT x 33 weeks: treatment resulted in fewer globules than at baseline and prevented inflammation

Z-AAT globule measurements (PAS-D)



- Globule number 42% less in PiZ mice treated with ARC-AAT (8 mpk) relative to baseline.
- Globule size in ARC-AAT treated mice was 2.75-fold smaller than in age-matched saline controls.
- Area of liver containing globules was 2.5-fold less in ARC-AAT treated mice than saline controls.

Liver expression of genes involved in AATD disease

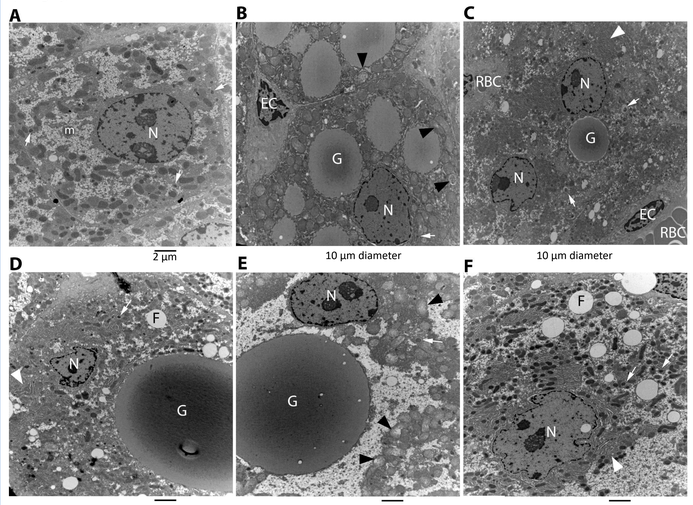


- ARC-AAT prevented increases in fibrosis gene expression (Col1a1, Mmp12, Mmp13, Timp1 and others not shown), approximately comparable to wild-type (WT) mice of similar age (w, weeks).
- Expression of stress, autophagy and apoptosis genes (Gpnmb, Nupr1) and redox-regulation genes (Cbr3 and GstM3) normalized.

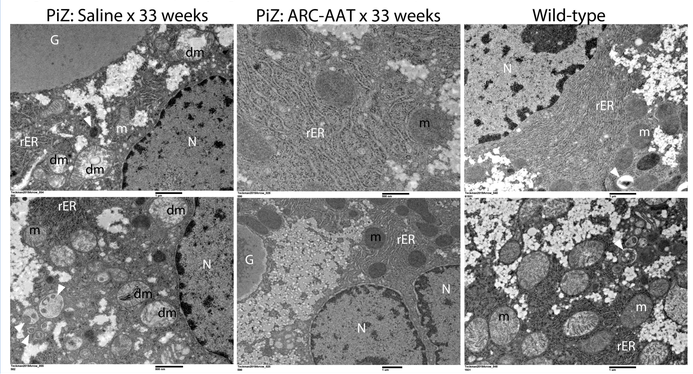
Ultrastructural imaging of mouse livers (electron microscopy)

Panels **A**: WT mouse (11 months old); **B,C**: PiZ, baseline; **D-F**: PiZ, saline x 33 weeks; **G-I**: PiZ, ARC-AAT x 33 weeks

Key: N, nucleus; G, globule; m, healthy mitochondria; F, microvesicular fat; RBC, red blood cell; EC, epithelial cell; black arrowheads, damaged mitochondria; white arrowheads, ER; white arrows, autophagosomes; glycogen stains pale grey/white. Scale bar = 2 µm



Key for close-up images below: N, nucleus; G, globule; m, healthy mitochondria; dm, damaged mitochondria; rER, rough ER; white arrowheads, autophagosomes. Scale bars 500 nm – 1 µm



Results of ultrastructural evaluation

- PiZ, baseline:
  - Numerous globules
  - Dilated endoplasmic reticulum
  - Many depolarized and damaged mitochondria
  - Increased autophagosomes
- PiZ, Saline x 33 weeks:
  - Very large globules
  - Abundance of dilated ER
  - Few healthy mitochondria
  - Many damaged mitochondria
  - Increased autophagosomes
  - Microvesicular fat within hepatocytes indicates unhealthy cells
- PiZ, ARC-AAT x 33 weeks:
  - Many healthy mitochondria
  - ER resembles that of healthy wild-type liver despite some residual small globules
  - Cellular architecture similar to healthy wild-type liver

CONCLUSIONS

Sustained RNAi reduction of Z-AAT reversed the AATD disease phenotype:

- Deeply reduced monomeric Z-AAT protein in the liver, reflected by deeply reduced plasma Z-AAT protein.
- Reduced polymeric Z-AAT in the liver.
- Prevented the dramatic increase in globule size seen in age-matched control PiZ mice.
- Restored normal ER.
- Prevented inflammation.
- Prevented/reduced expression of fibrosis, redox-regulation, stress, apoptosis and autophagosome-associated genes.
- Resulted in abundance of healthy mitochondria

RNAi holds great promise for the treatment of patients with AATD-associated liver disease. Next generation subcutaneously-administered RNAi therapeutic ARO-AAT is now in Phase 2/3 clinical trials.

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