RNAi therapeutic ARC-AAT prevents production of Z-alpha1 antitrypsin polymers and reverses liver disease phenotype in PiZ mouse model

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arrowhead pharmaceuticals

Disclosures

Employed by Arrowhead Pharmaceuticals

Alpha-1 antitrypsin deficiency

- AATD is a large scale orphan disease
  - Alpha-1 Foundation estimates 100,000+ in the US
  - Approximately 100,000+ in Europe
- Mutation in AAT gene (Z-AAT) leads to mis-folding of the protein and poor export from hepatocytes: low levels in circulation and accumulation in liver

Pathophysiology

Lung

Tissues susceptible to damage by neutrophil proteases: COPD

Treated with AAT enzyme replacement therapy

Liver

Accumulation of mutant Z-AAT protein can cause cirrhosis and HCC

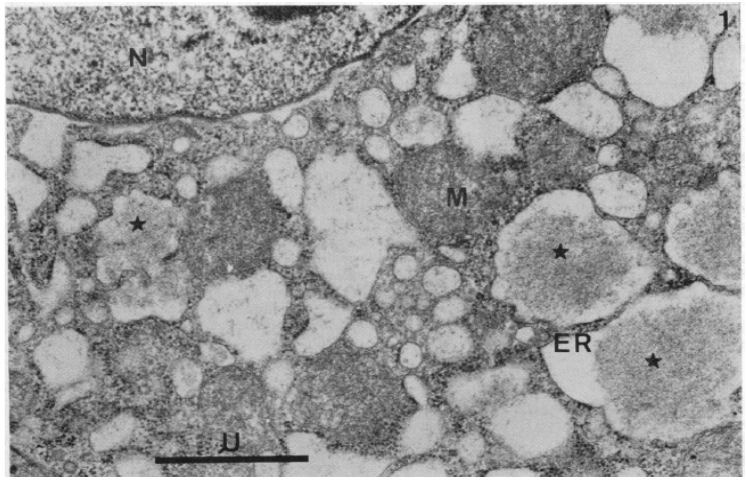
Currently no treatment

ARC-AAT mechanism of action

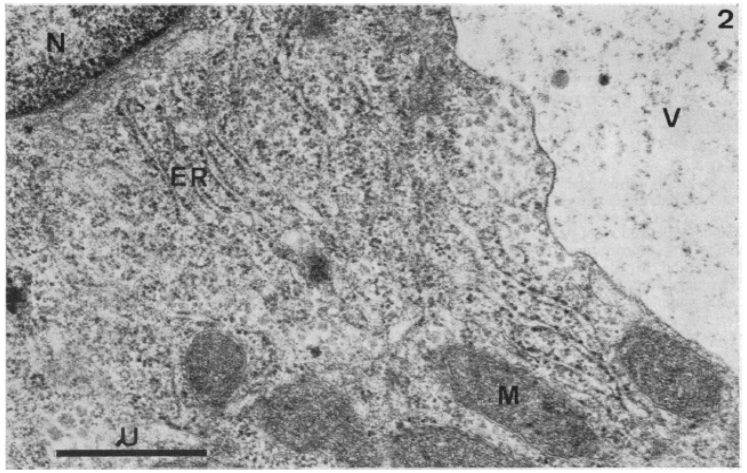
ARC-AAT designed to stop Z-AAT production by silencing AAT gene to:

- Prevent accumulation of disease-causing protein in liver
- Allow clearance of accumulated protein
- Prevent repeated cycles of cellular damage
- Reverse fibrosis associated with prior damage

PiZZ phenotype (diseased)



Pi null phenotype (normal)

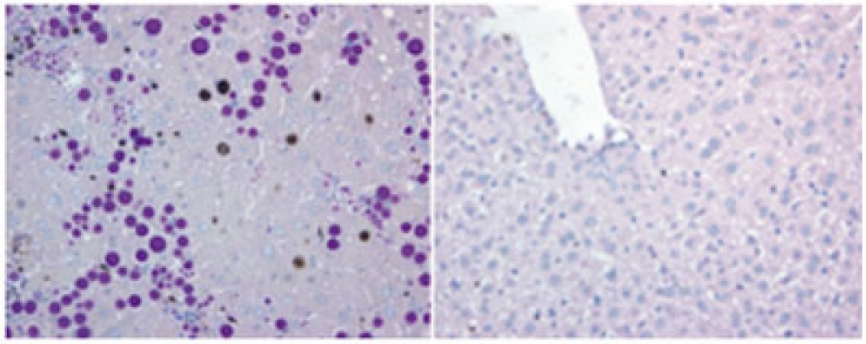


Feldmann G et al., *Gut* 1975

AAT deficiency liver disease mouse model

The transgenic PiZ mouse model expressing the human Z-mutant AAT gene (Z-hAAT) recapitulates the human AATD-associated liver phenotype:

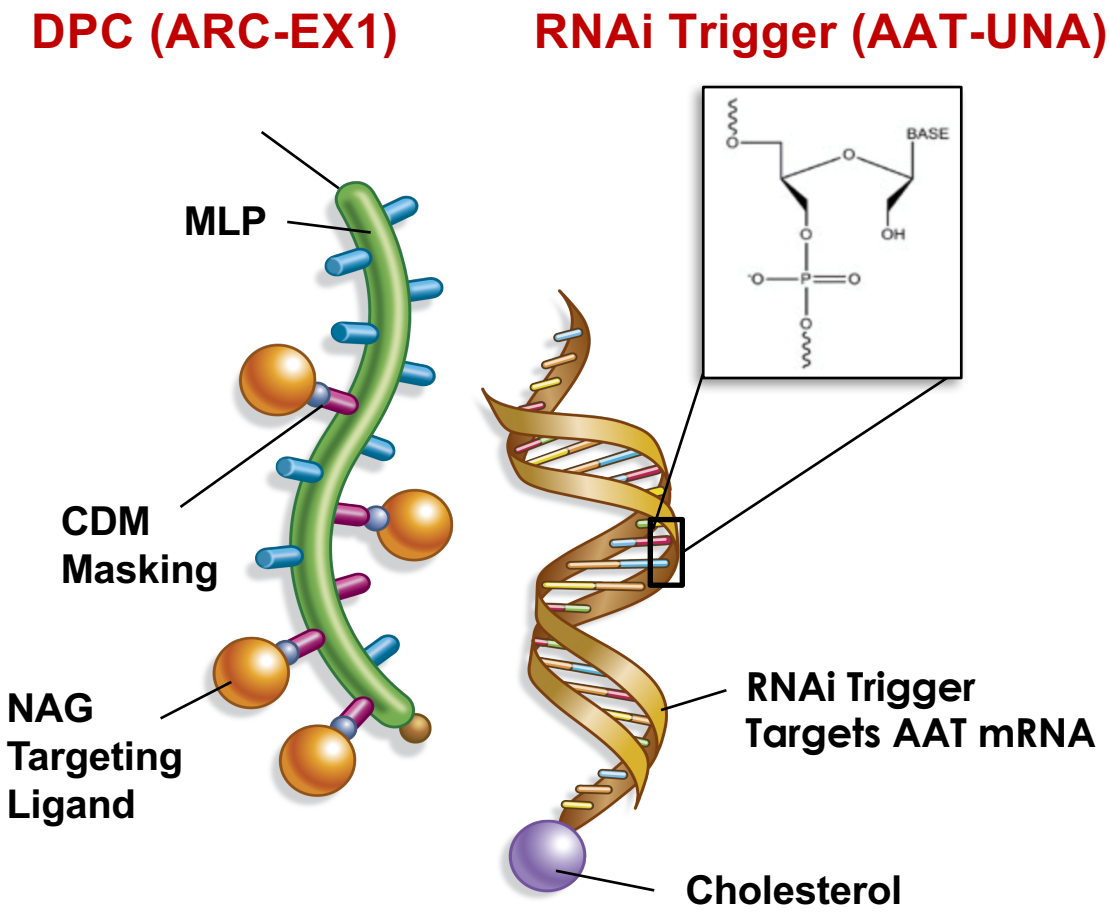
- Hepatocytes produce high levels of human Z-hAAT
- Hepatocytes are not able to efficiently process and secrete the Z-hAAT
- Z-hAAT forms polymers that accumulate in large "globules" within the hepatocytes
- Presence of polymer stresses hepatocytes, eventually leading to HCC
- Globules are visualized with Periodic Acid Schiff (PAS) staining + diastase



PiZZ **Wild Type**

Rudnick DA et al., *Hepatology*. 2004

ARC-AAT: An RNAi therapeutic for AATD-associated liver disease using Dynamic PolyConjugate (DPC) technology



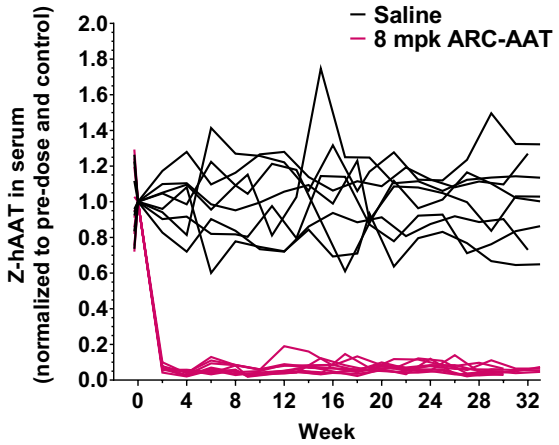
MLP: melittin-like peptide
CDM: carboxy-dimethylmaleamide
NAG: N-acetyl-galactosamine

Efficacy of ARC-AAT in PiZ mouse model

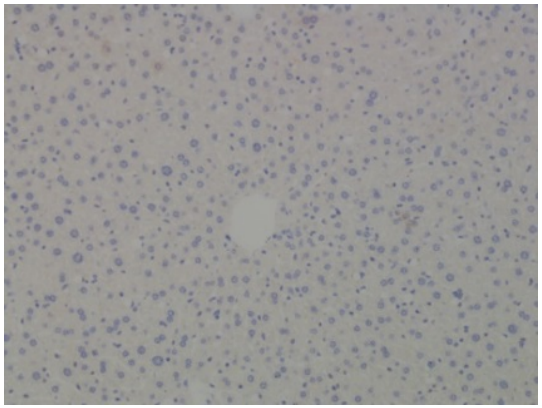
Study design

- **Objectives:**
  - Improve liver disease phenotype of treated animals relative to same age controls
  - Reduce/reverse phenotype observed at baseline
- **Groups**:
  - Baseline, assessed on Day 1
  - ARC-AAT (AAT-UNA + DPC delivery reagent), Q2W for 32-33 weeks
  - Negative controls (Saline, AAT-UNA alone), Q2W for 32-33 weeks
- **Evaluation**:
  - Z-hAAT protein (ELISA, Western blot, PAS-D)
  - Liver histology
  - Gene expression associated with disease

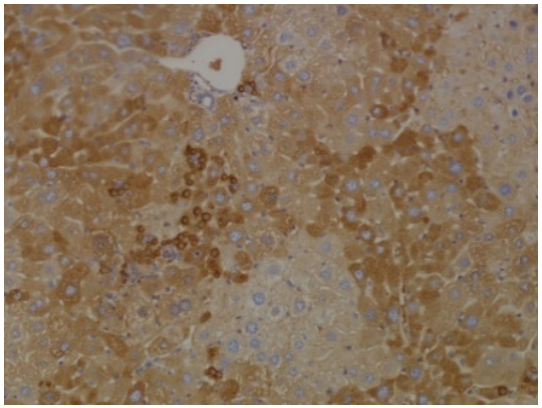
Reduction of Z-hAAT in PiZ mice



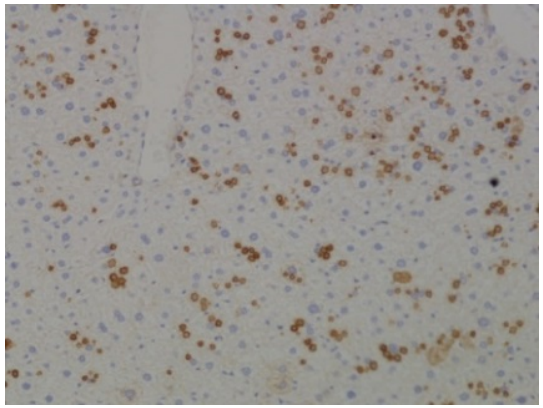
- Serum Z-hAAT reduced >90% by ARC-AAT treatment
- Z-hAAT cleared from the cytoplasm by ARC-AAT treatment



Negative Control
Wild-type mouse

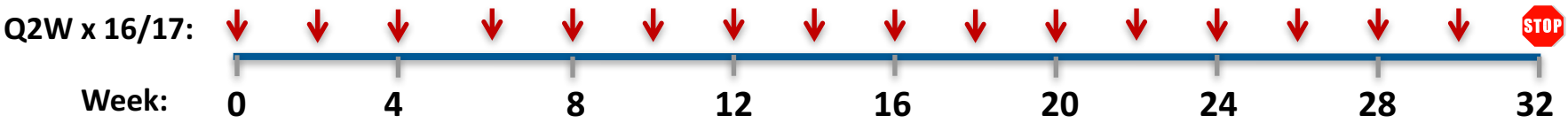


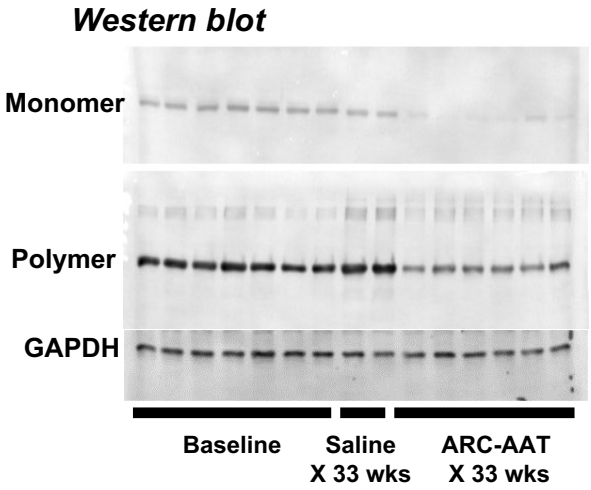
Saline Control – 33 weeks
PiZ mouse

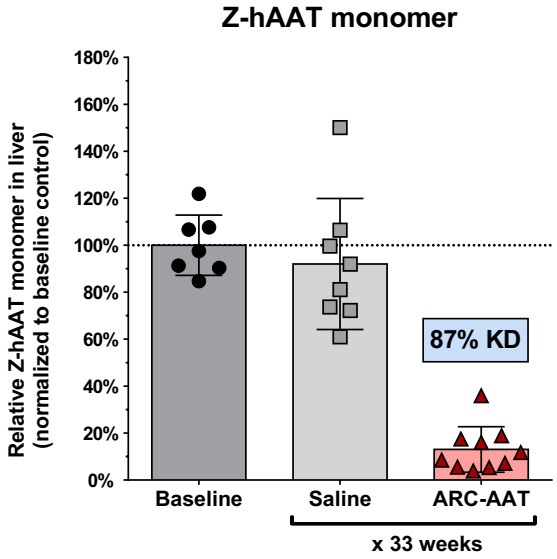


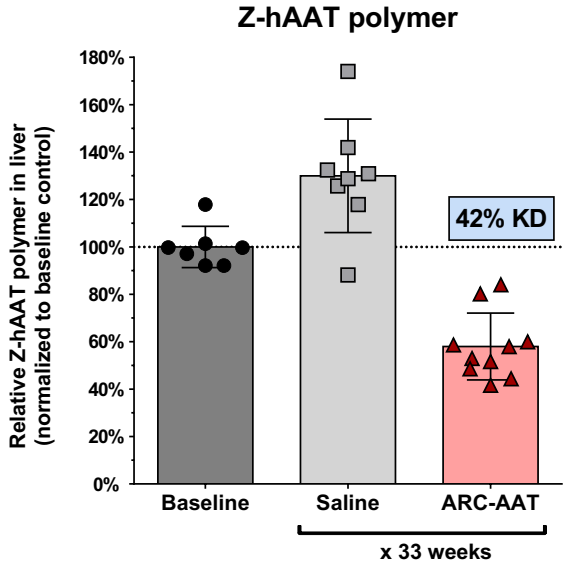
ARC-AAT – 33 weeks
PiZ mouse

ARC-AAT reduces polymeric Z-hAAT in liver



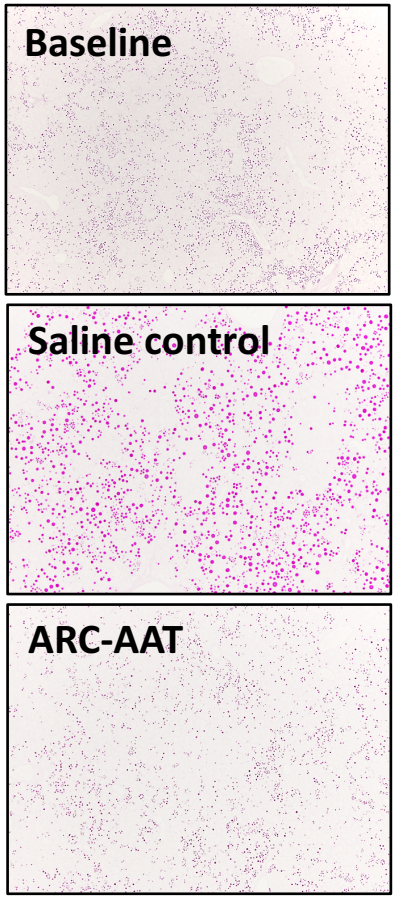




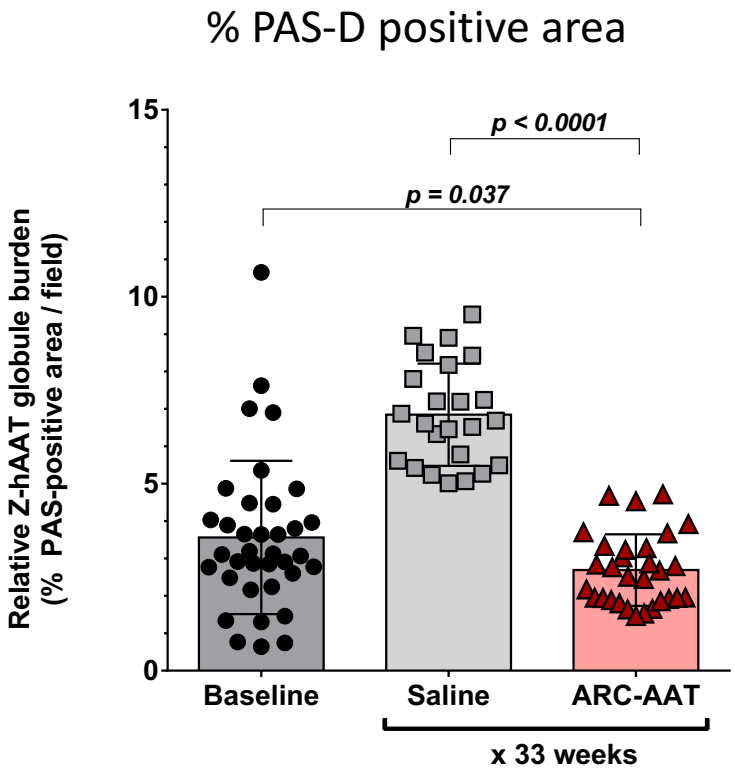


Prevention and reversal of the polymer accumulation

ARC-AAT halts accumulation of Z-hAAT globules in liver

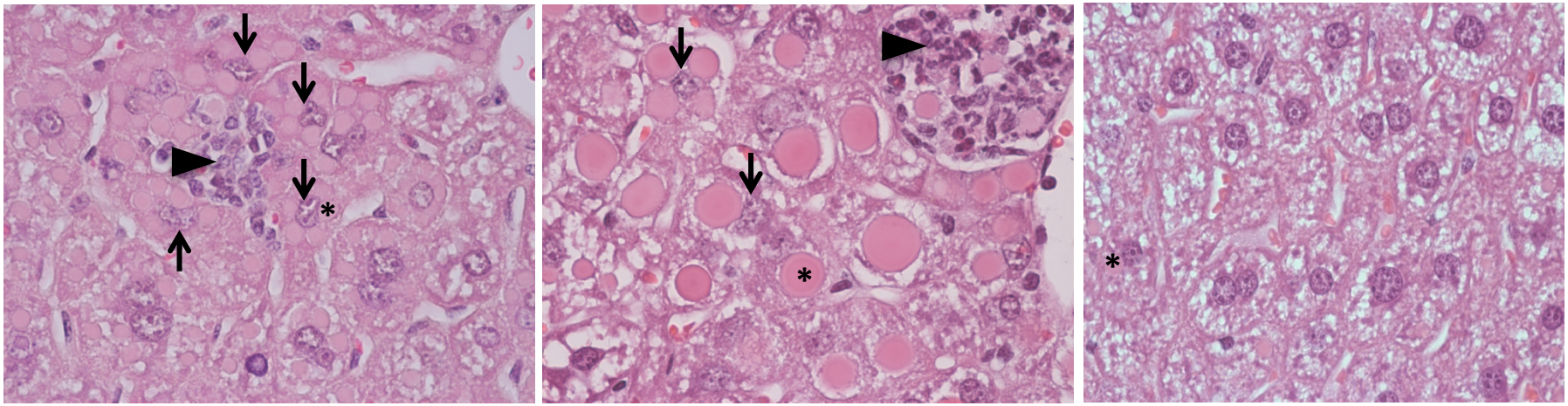


PAS-D stained Z-hAAT globules



PAS-D positive area 61% less in ARC-AAT treated compared to saline controls and 24% less than at baseline

Improved histopathology following ARC-AAT treatment



Male PiZ mice
H&E stained liver sections, x1000

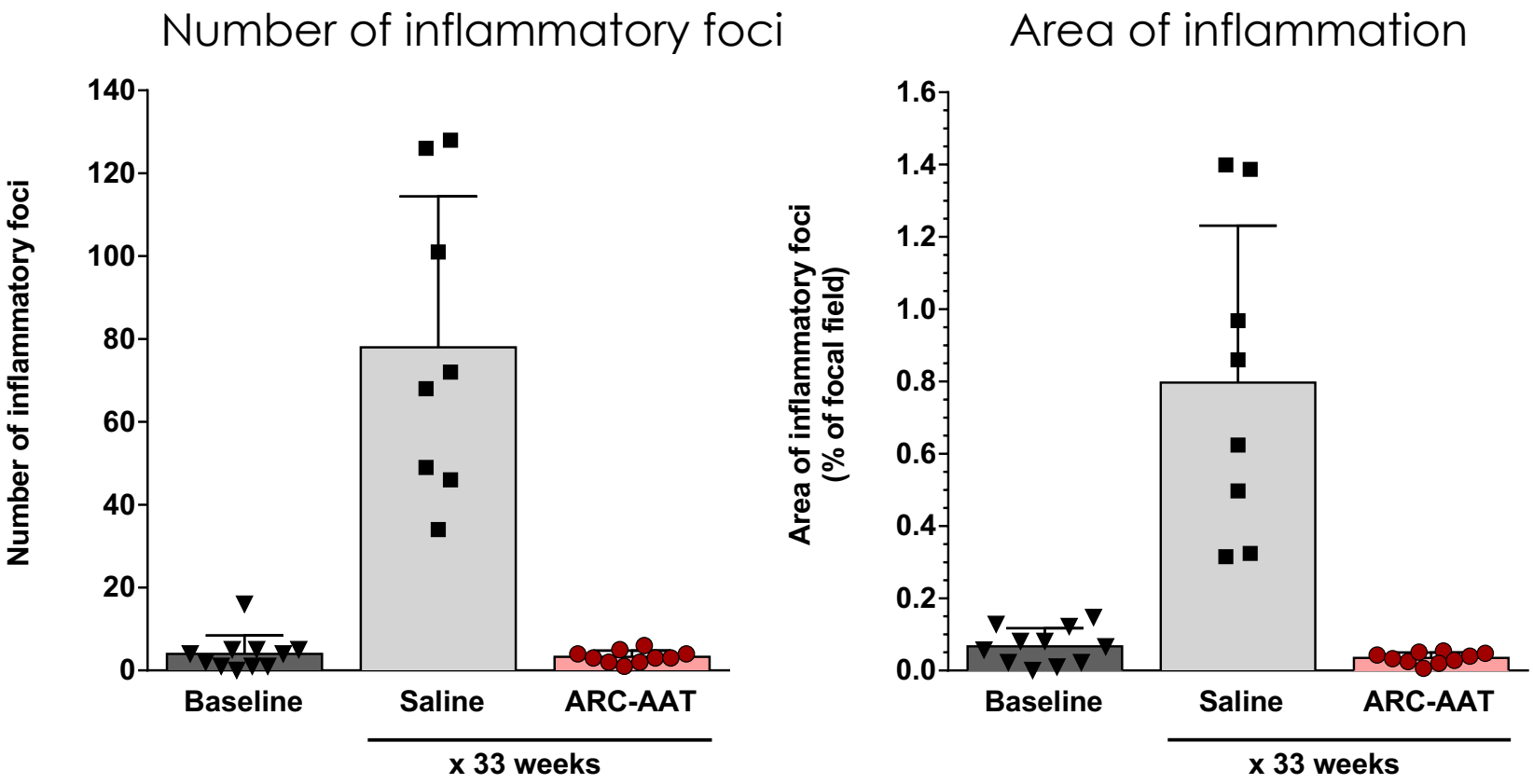
Baseline (11-17 weeks old)
- Significant globule accumulation (*);
- compressed nuclei (black arrows);
- apoptosis & inflammatory cells (arrowhead)

Saline control (Q2W x 33 wks)
- Significant globule accumulation (*), size 25-35 µm;
- compressed nuclei (black arrows);
- inflammatory cells (arrowhead)

8 mpk ARC-AAT (Q2W x 33 wks)
- Minimal to moderate globule accumulation (*), size 7-10 µm; no compressed nuclei,
- no inflammatory cells

ARC-AAT treatment improves liver health and prevents further damage

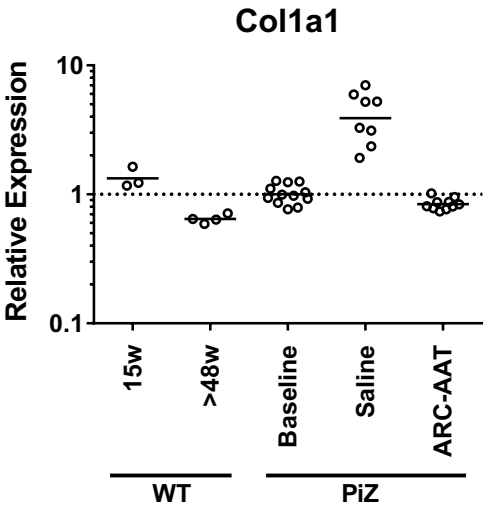
Prevention of inflammation in the liver

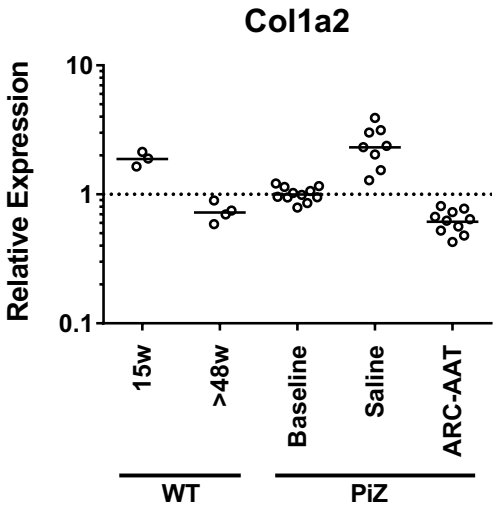


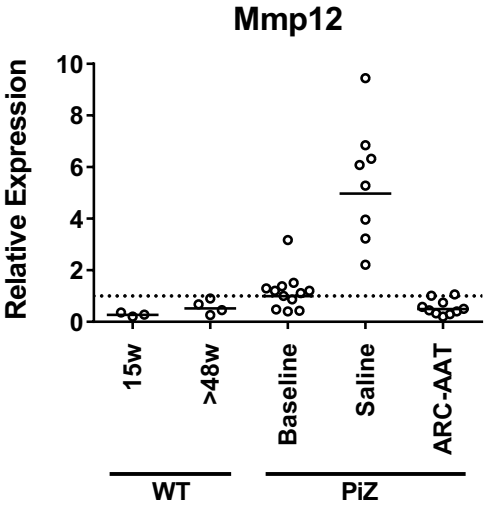
ARC-AAT treatment prevented inflammation

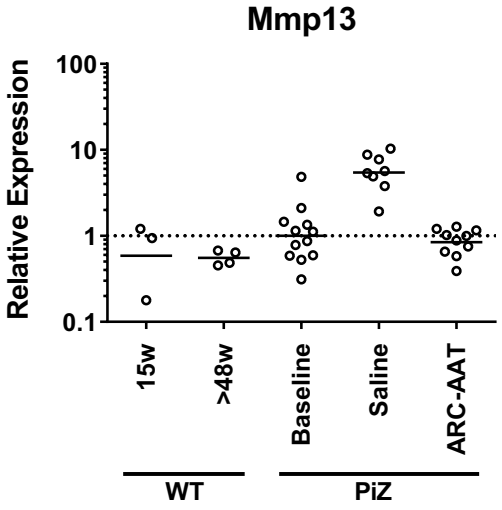
- Fewer inflammatory foci
- Reduced total area of inflammation

Reduced gene expression associated with fibrosis in the liver



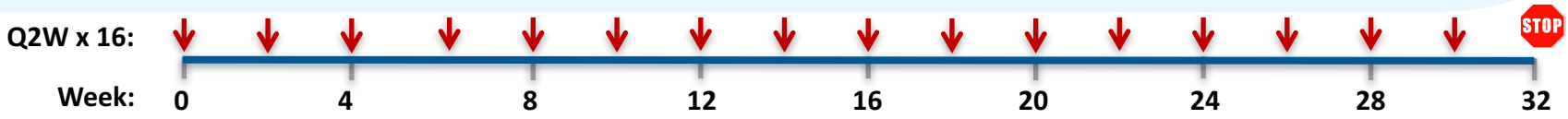


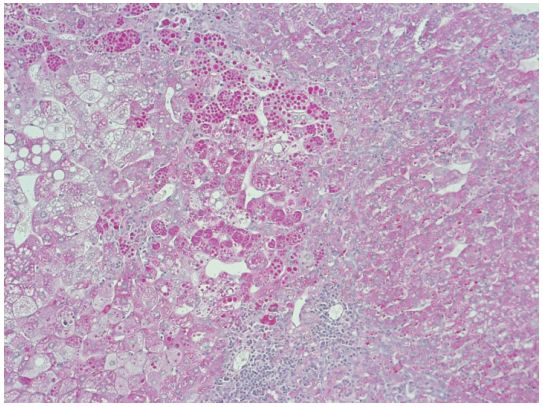




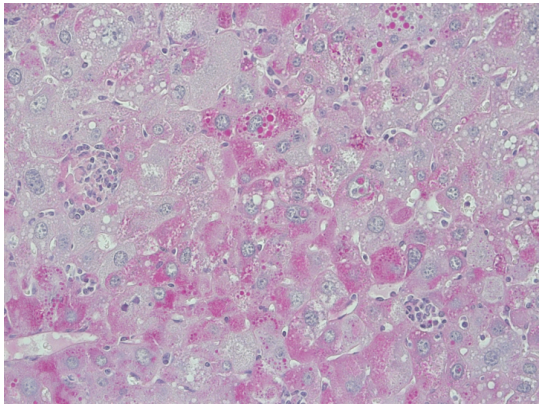
- Fibrosis gene expression increases with age in untreated (saline group) PiZ mice
- ARC-AAT prevents the increase in fibrosis gene expression

Prevention of liver tumors in PiZ mice

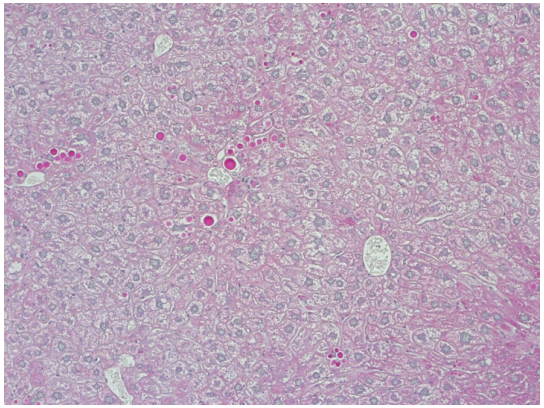




Baseline (15-16 months old)
PAS-D globules, inflammation, neoplastic hepatocytes in some mice

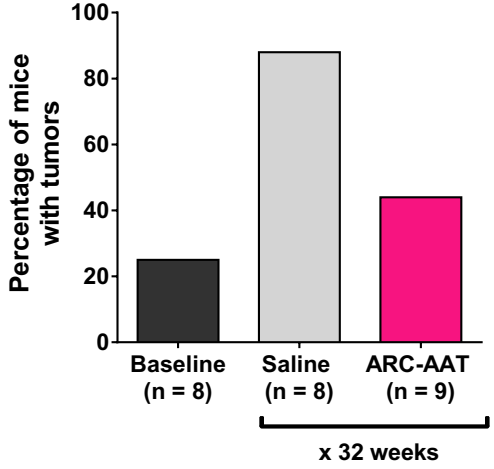


Saline x 32 weeks
PAS-D globules, inflammation, neoplastic hepatocytes, tumors



ARC-AAT x 32 weeks
Rare PAS-D globules, normal morphology

- Some mice had tumors and/or neoplastic hepatocytes at baseline that increased tumor incidence over time
- ARC-AAT reduced tumor incidence over the treatment period



ARC-AAT: Summary

- AATD is a large scale and underdiagnosed orphan disease affecting 100,000+ individuals in the U.S.
- Replacement enzyme therapy for the lung disease allows longer survival of patients who as a result increasingly manifest hepatic disease: cirrhosis and hepatocellular carcinoma
- Repeat injection of ARC-AAT in transgenic PiZ mice
  - Reduced Z-hAAT polymers
  - Prevented inflammation
  - Normalized gene expression associated with liver disease
  - Prevented tumors
- ARC-AAT is now in clinical trials with PiZZ patients

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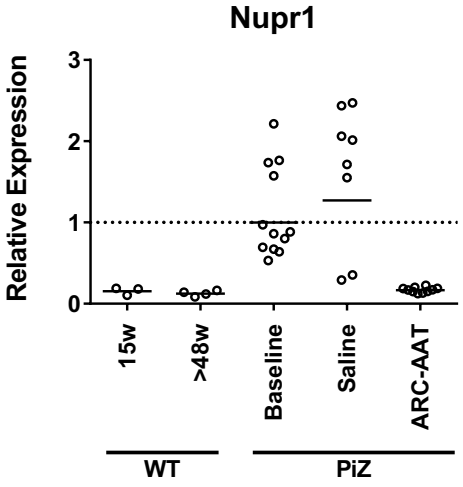
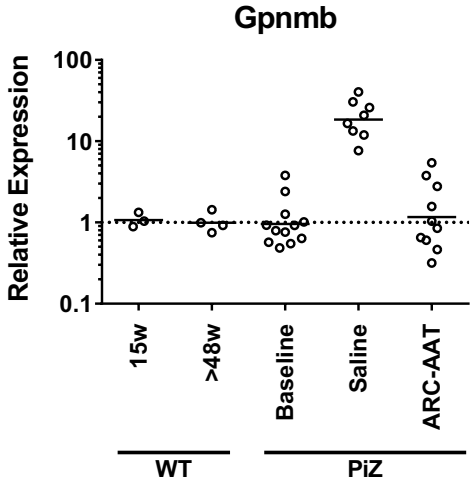
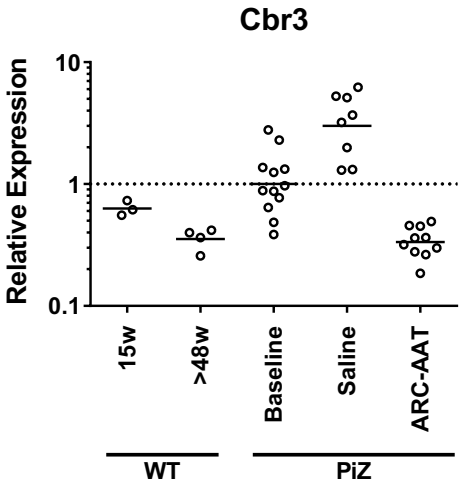
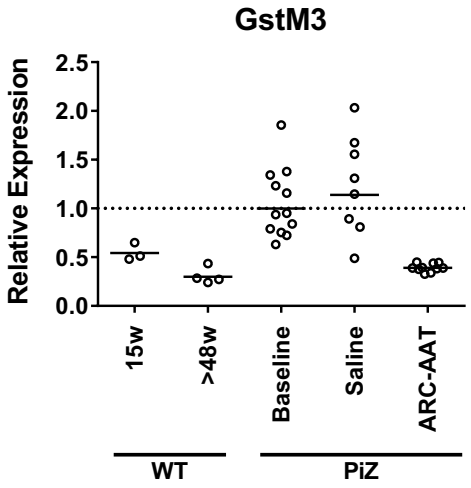
Normalization of stress response, proliferation and redox gene expression









Nupr1 – nuclear protein, transcriptional regulator 1; chromatin binding protein that converts stress signals into program of gene expression

Gpnmb - transmembrane glycoprotein NMB; involved in cell proliferation

Cbr3: carbonyl reductase (NADPH) 3, NADPH-dependent oxidoreductase

GstM3: glutathione-S transferase