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A Hepatocyte-Targeted RNAi Therapeutic for Alpha1-Antitrypsin Deficiency-Associated Liver Disease

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Christine I. Wooddell, PhD
Group Leader – Arrowhead Research Corporation

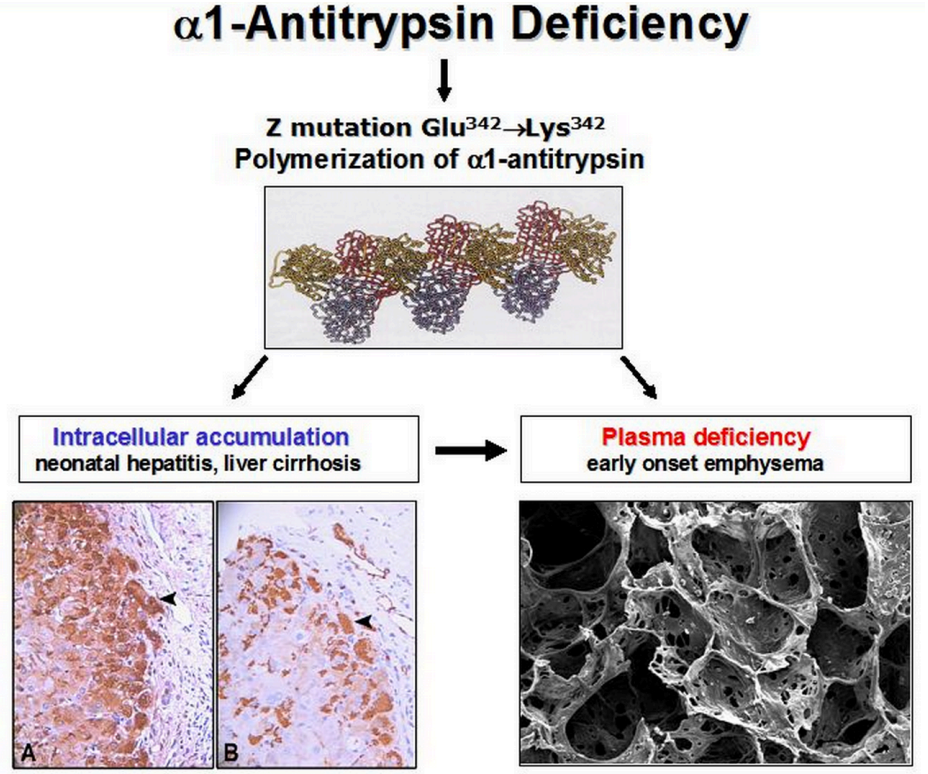
Alpha-1 antitrypsin deficiency (AATD)-associated liver disease

- Alpha-1 antitrypsin is a glycoprotein protease produced predominantly (~90%) by the liver and secreted into the serum.
- The primary function of AAT is to inhibit neutrophil elastases in the lungs, primarily during times of inflammation.
- AATD is due to misfolded AAT of the Z-allele, called Protease inhibitor Z (PiZ), which causes an autosomal co-dominant disease.
- The misfolded Z-AAT is poorly secreted from hepatocytes in the liver, although continually produced at high levels, resulting in accumulation of the misfolded protein. The Z-AAT forms polymers in the endoplasmic reticulum of the hepatocytes.
- Most individuals with AATD are homozygotes for the PiZ allele (~1 in 3000 births in the U.S. are PiZZ).

Alpha-1-antitrypsin deficiency (AATD): A protein folding, gain-of-function liver disease

- 95-98% of the clinical disease from Z-mutant homozygosity (PiZZ)
- ZZ mutation leads to less secreted protein with lower activity
- Polymer formation and retention in hepatocyte ER

	Phenotype				
Units	PI*MM	PI*MZ	PI*SS	PI*SZ	PI*ZZ
μM	20–48	17–33	15–33	8–16	2.5–7
mg/dl	150–350	90–210	100–200	75–120	20–45



Globule formation → fibrosis → cirrhosis → HCC

Janciauskiene, S. et al. (2013) *Acute Phase Proteins*

Alpha-1 antitrypsin deficiency

- AATD is a large scale orphan disease
  - Alpha-1 Foundation estimates 100,000 + in the US
  - Similar number in Europe
- Mutation in AAT gene 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 enzyme replacement therapy

Liver

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

↓

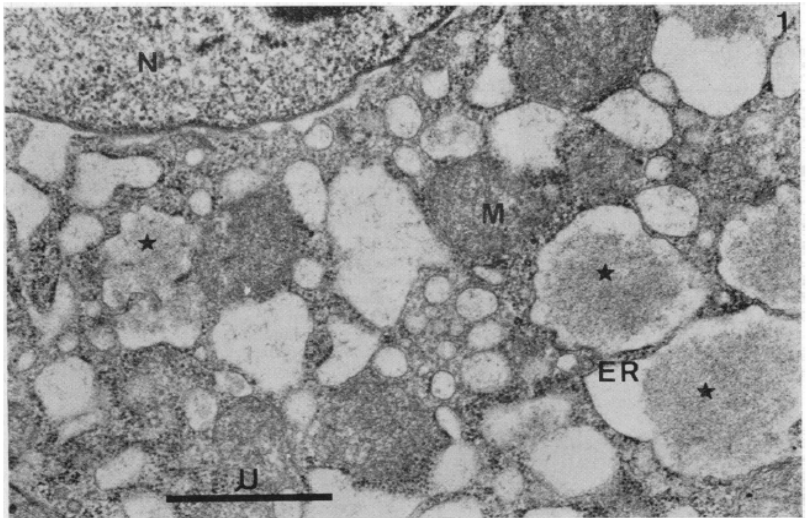
No current treatment

ARC-AAT rationale

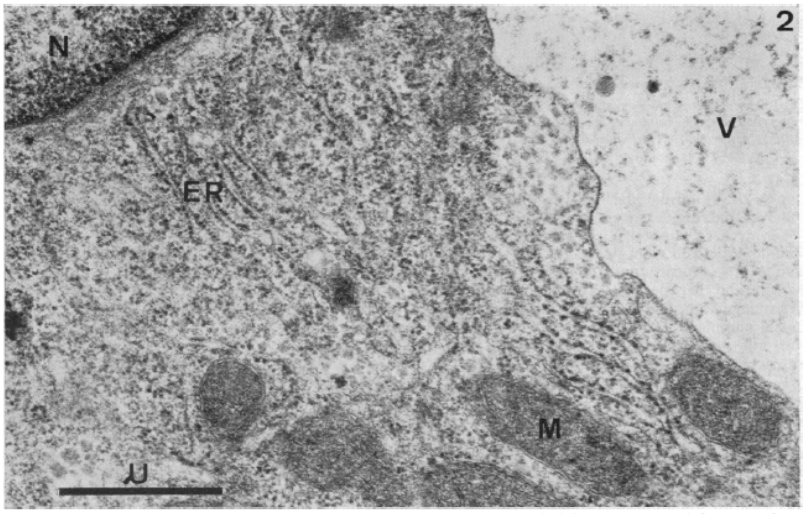
ARC-AAT designed to stop Z-AAT production by silencing AAT gene to:

- ***Prevent*** accumulation of disease-causing protein in liver
- ***Allow*** clearance of accumulated protein
- ***Prevent*** repeated cycles of cellular damage and liver tissue repair
- ***Reverse*** fibrosis associated with prior damage by allowing repair

PiZZ phenotype (diseased)



Pi null phenotype (normal)

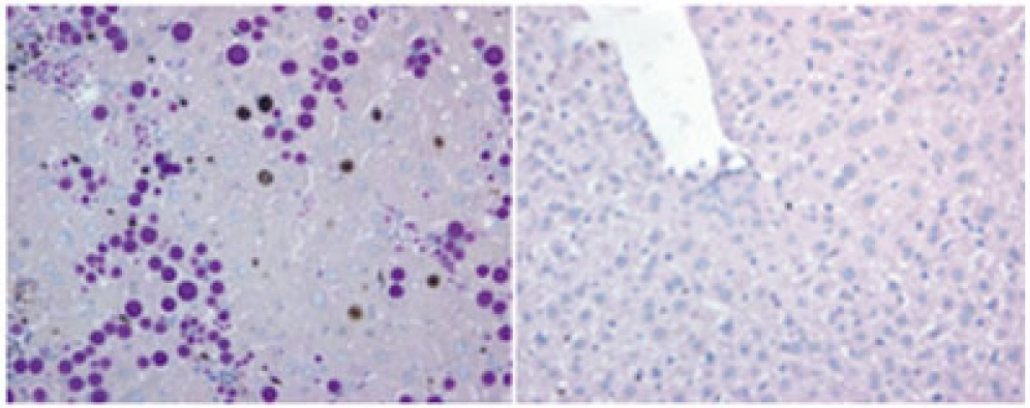


Feldmann G et al., *Gut 1975*

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
- These globules stress the hepatocytes, eventually leading to fibrosis and hepatocellular carcinoma



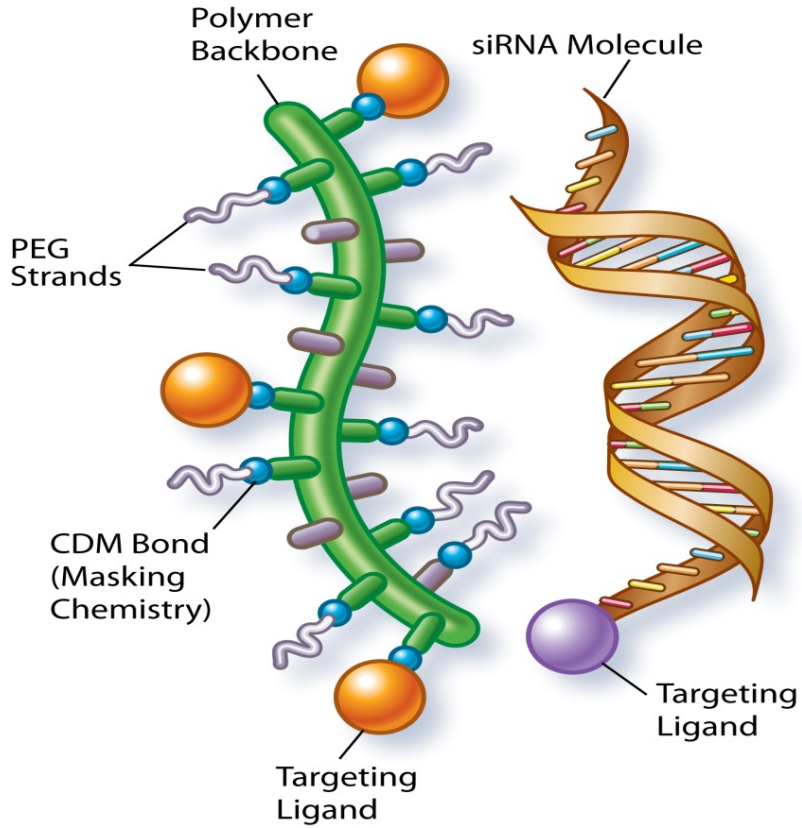
PiZZ **Wild Type**

Rudnick DA et al., *Hepatology. 2004*

Two component DPC technology for delivery of RNAi triggers

DPC

- Amphipathic polymer (or peptide) that promotes endosomal escape of RNAi trigger
- Polymeric amines “masked” with pH-labile moiety, unmasked in endosome
- Slightly negatively charged
- Targetable
- Co-injected with RNAi trigger



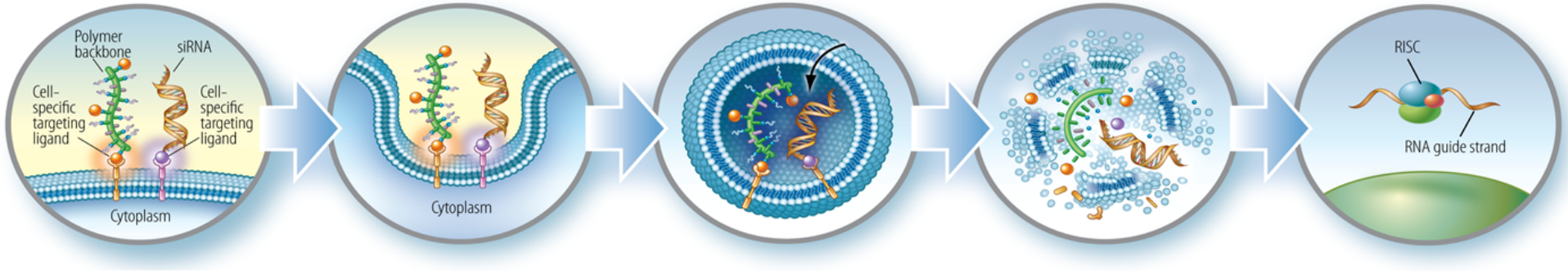
RNAi Trigger

- Canonical siRNA or other format
- Targeting ligand (eg. cholesterol, GalNAc) attached to sense strand

DPC and RNAi trigger do <u>NOT</u> form a complex,

they are separately targeted to the tissue of interest

RNAi therapeutics using Dynamic PolyConjugate (DPC) technology



DPC polymer and RNAi trigger (siRNA) attach to their respective cell surface targets

DPC polymer and RNAi trigger are internalized

DPC polymer and RNAi trigger are enclosed in endosomes. Low pH results in polymer unmasking

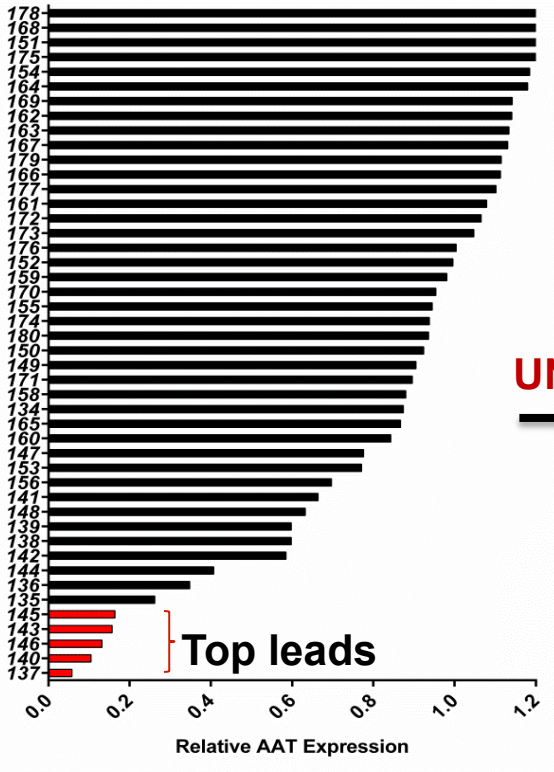
DPC polymer induces endosome disruption and release of RNAi trigger into cytoplasm

RNAi trigger engages RISC resulting in target knockdown

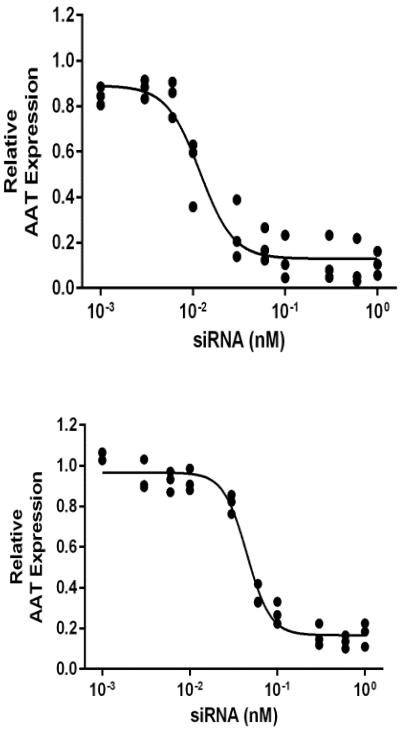
1. Rozema et al. (2007) Proc Natl Acad Sci USA 104:12982-12987
2. Wong et al. (2012) Nucleic Acid Ther 22:380-390
3. Wooddell et al. (2013) Mol Ther 21:973-985

Screening for RNAi triggers *in vitro* and *in vivo*

Human hepatocyte cell line

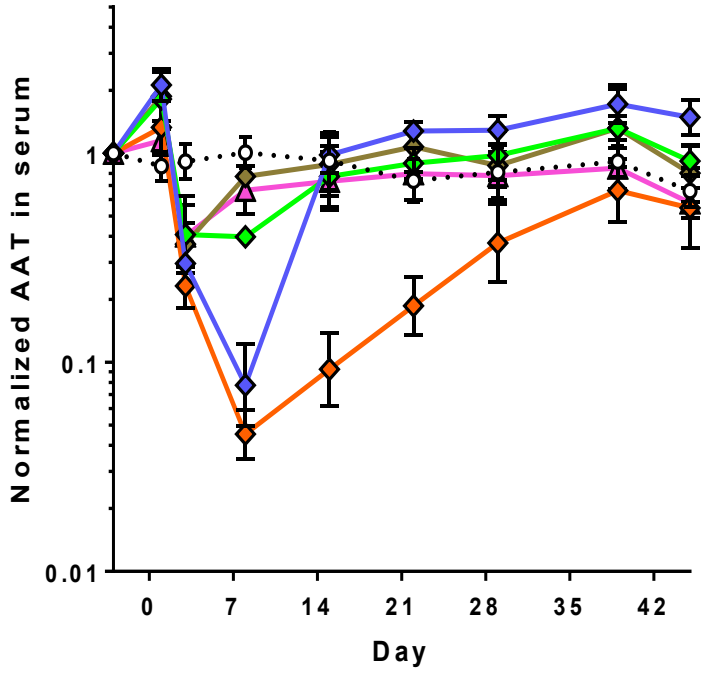


UNA →



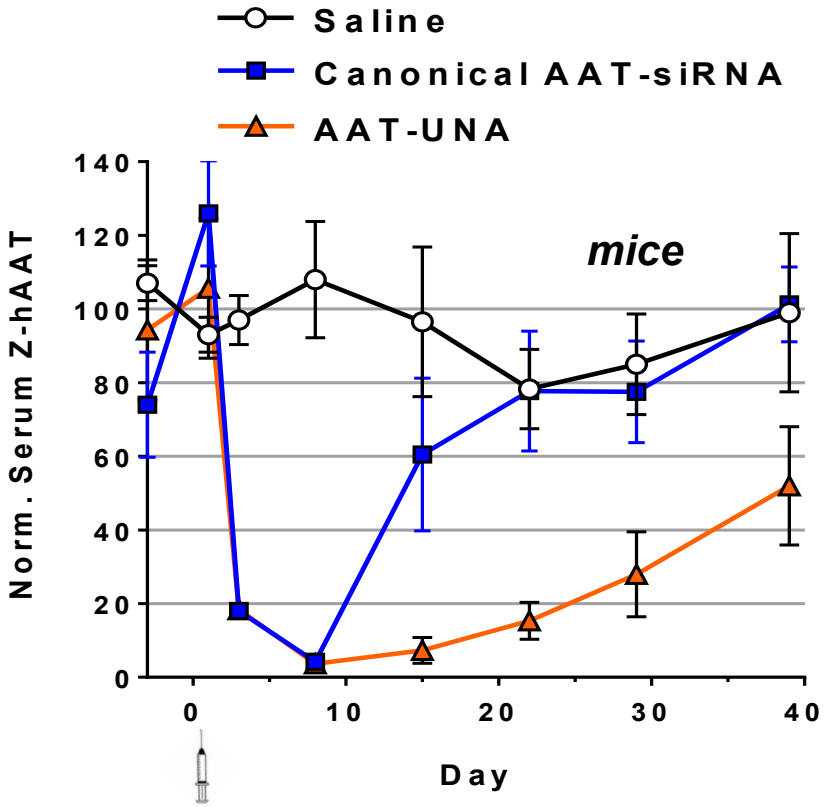
Chol →

Z-hAAT transgenic mice (PiZ)

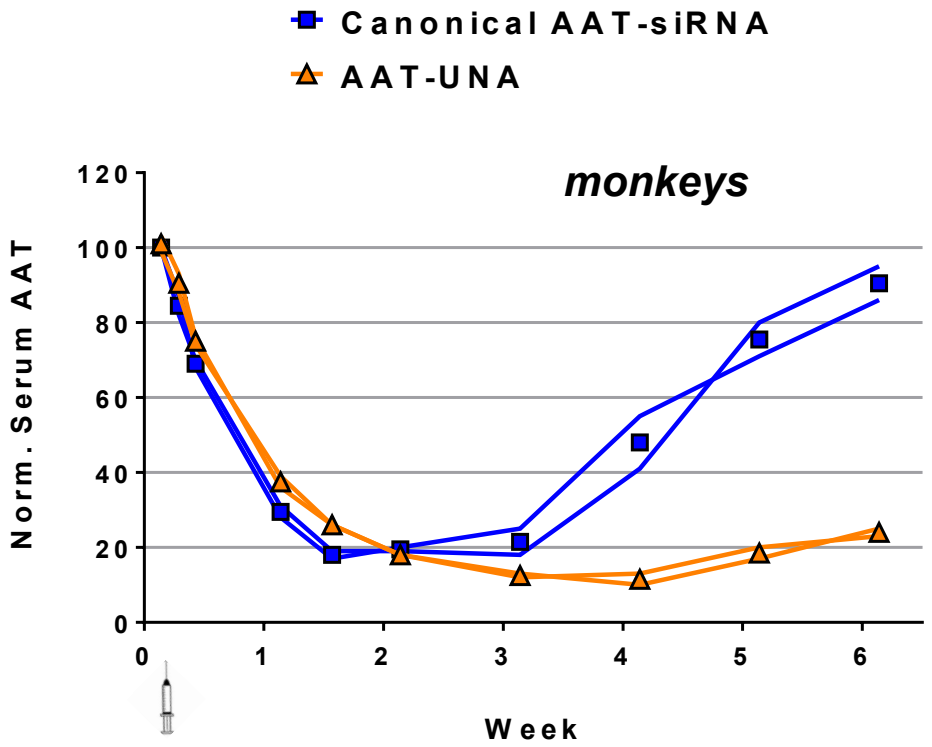


- 2,648 19-mer sequences identified, 840 human/cyno monkey cross-reactive, selected for in vitro screening after application of specificity criteria 46
- EC_{50} values for highly potent UNA-containing sequences identified for ranking
- Screening of top Chol-RNAi triggers + ARC-EX1 in transgenic Z-hAAT mice

Incorporation of a single UNA in AAT RNAi trigger sequence results in improved KD duration



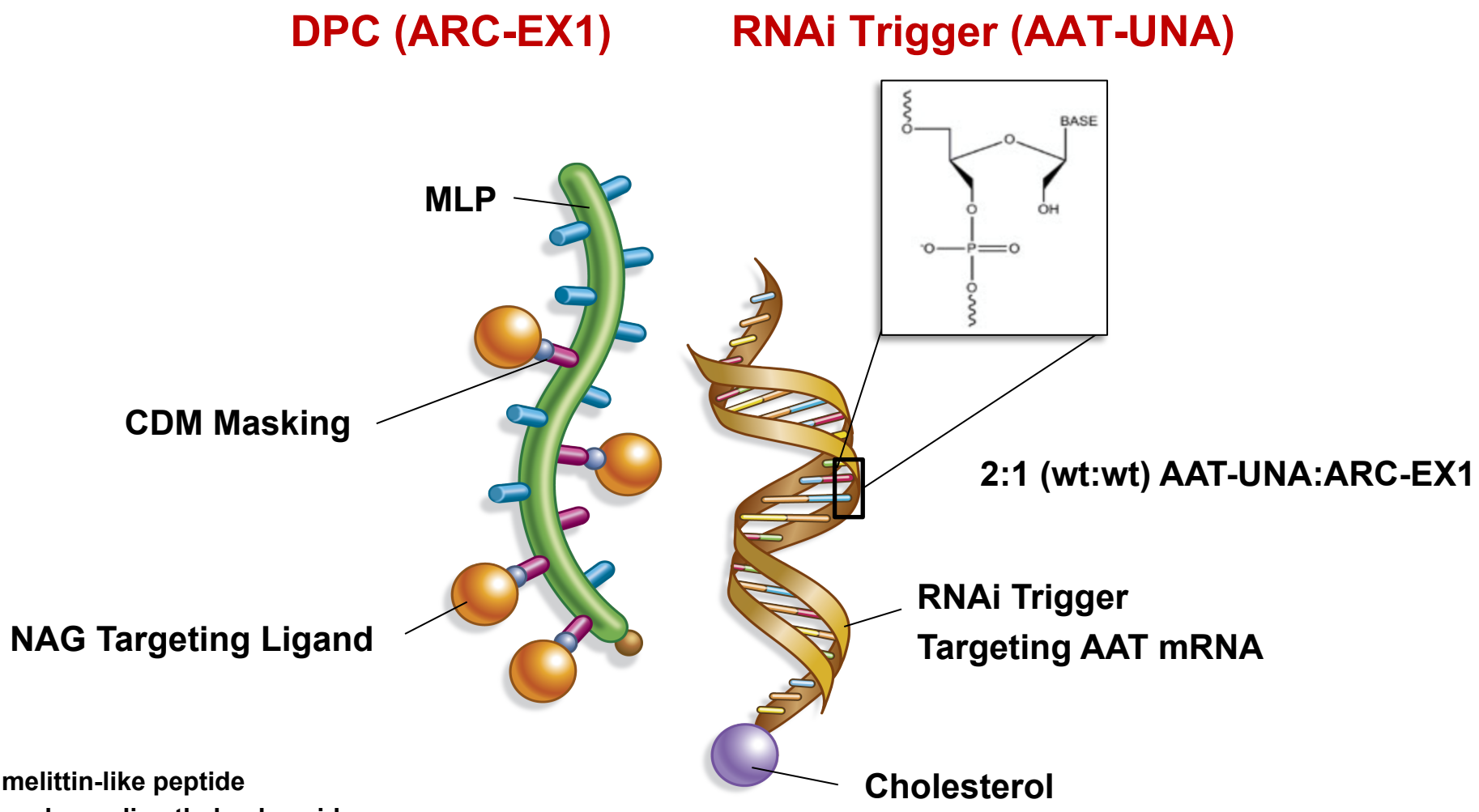
8 mg/kg RNAi trigger
+ 8 mg/kg ARC-EX1



6 mg/kg RNAi trigger
+ 3 mg/kg ARC-EX1

Max knockdown:	**UNA ≈ canonical siRNA**
Duration of effect:	**UNA >> canonical siRNA**

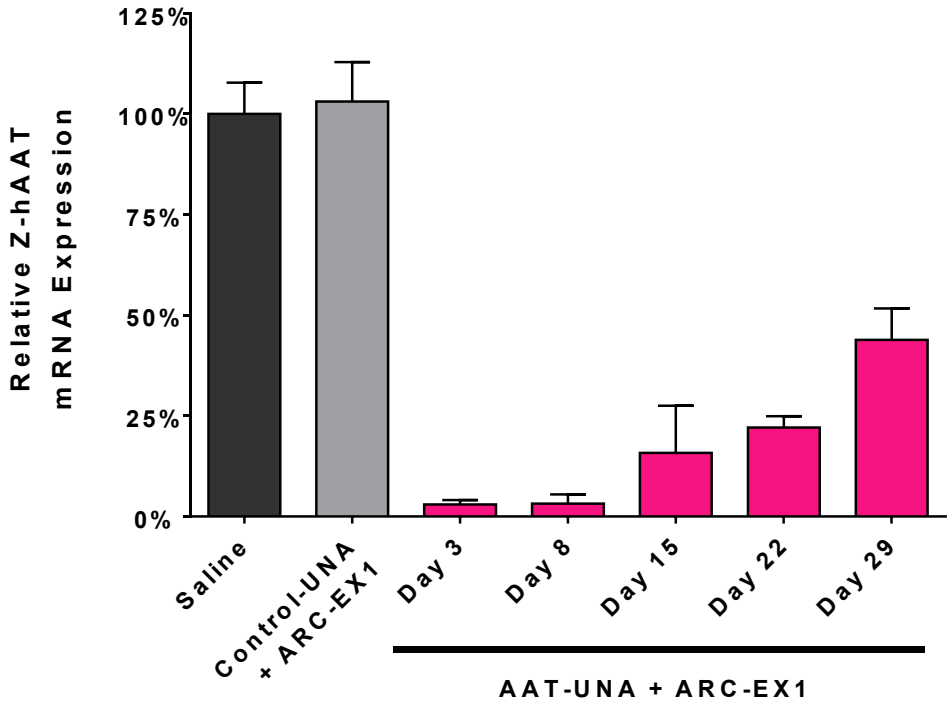
ARC-AAT: An RNAi therapeutic for AATD-associated liver disease using Dynamic PolyConjugate (DPC) technology

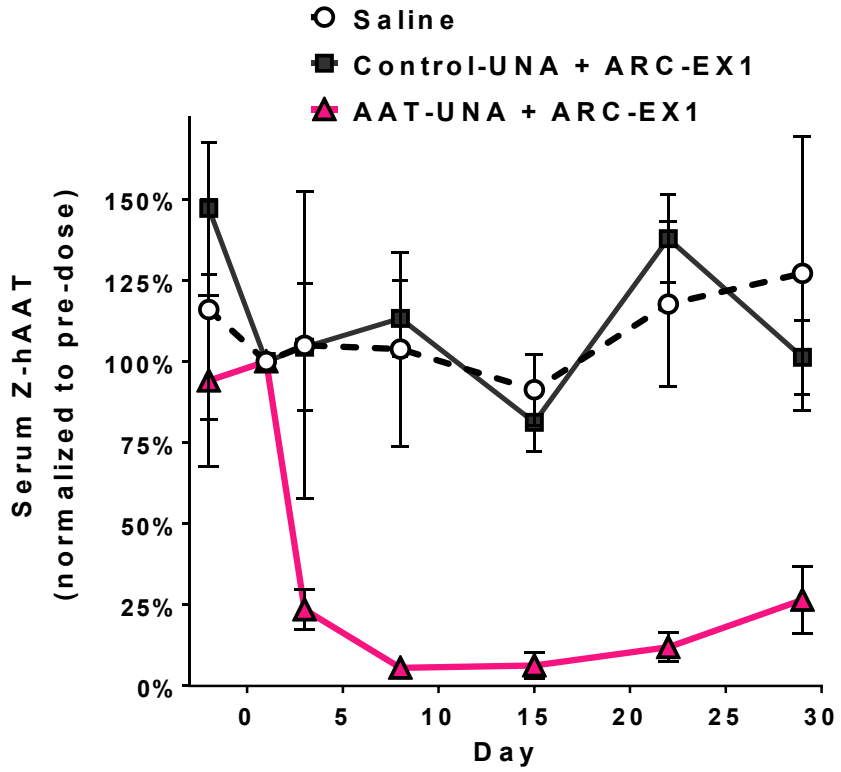


MLP: melittin-like peptide
CDM: carboxy-dimethylmaleamide
NAG: N-acetyl-galactosamine

Z-hAAT mRNA and serum protein reduction

Single injection, PiZ mice

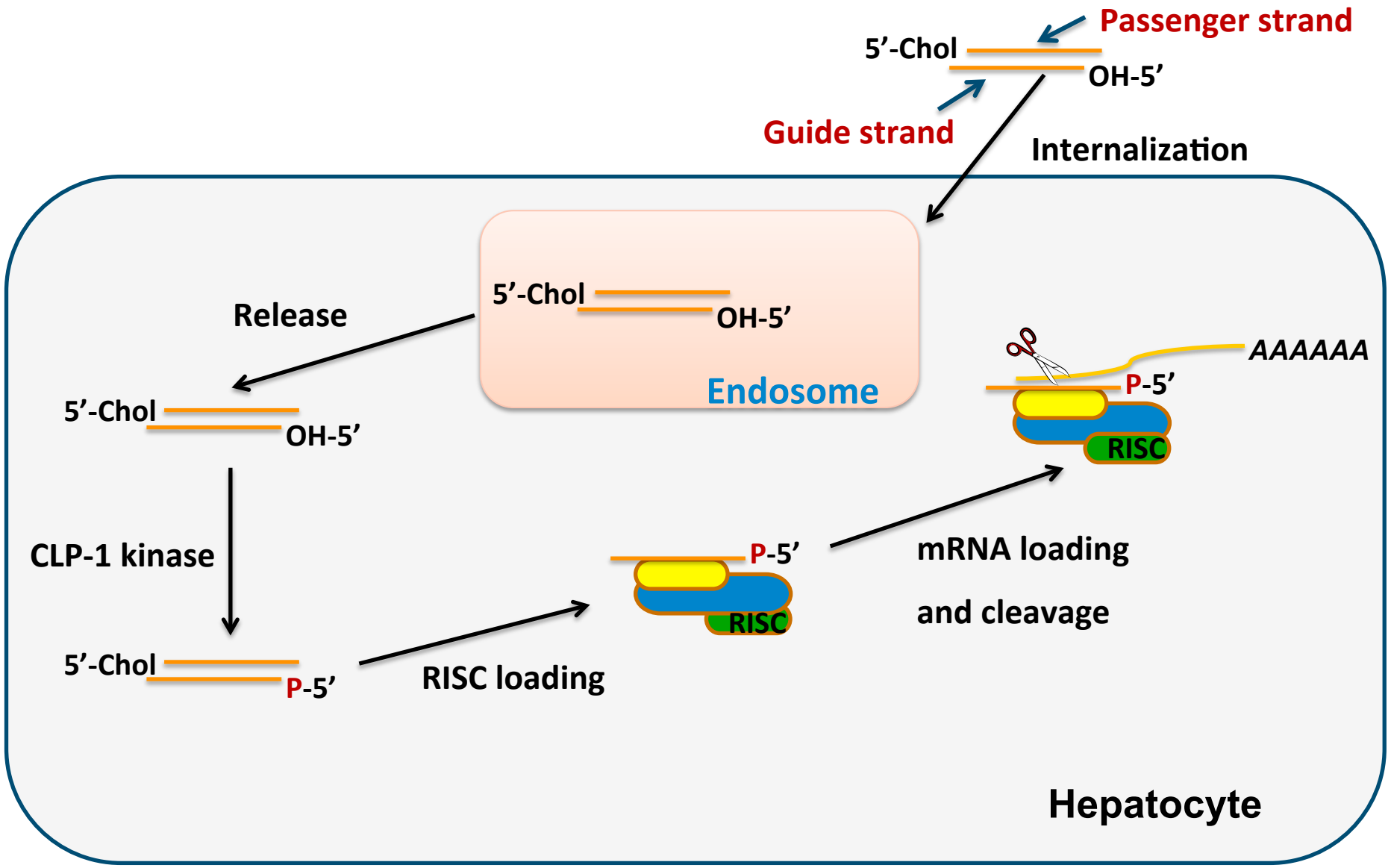




- **Z-hAAT mRNA and serum protein deeply reduced (~95%)**
- **Long duration of effect**

Female PiZ mice, 6 months old, 8 mg/kg AAT-UNA

Phosphorylation of trigger guide strand and RISC loading

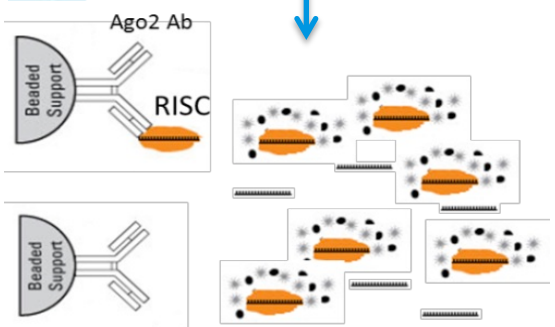


RNAi trigger in RISC exhibits long duration

- **Ago2 immunoprecipitation captures 5'-phosphorylated trigger; detected in PNA assay**
- **PiZ mice treated with single dose of ARC-AAT**

Homogenize liver (Total lysate)

Ago2 immunoprecipitation (IP)

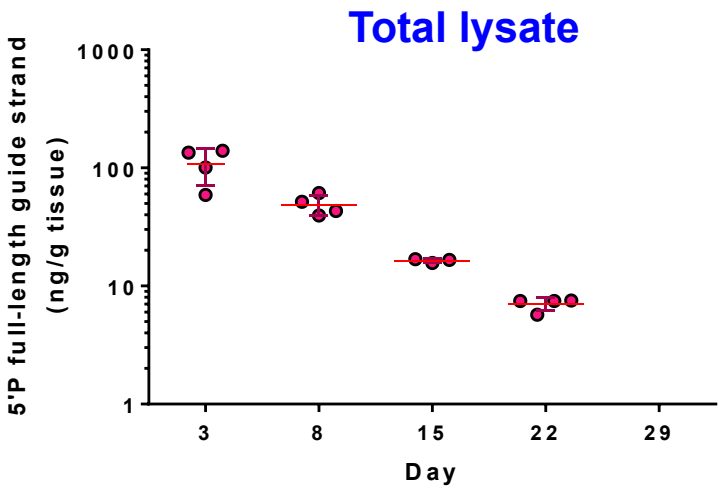


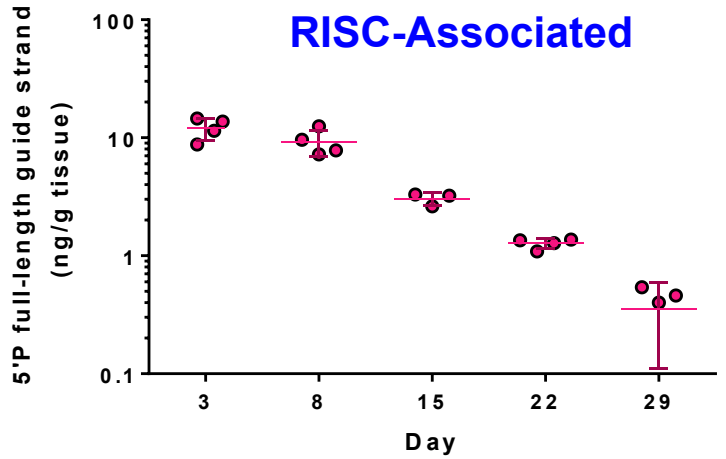
Wash

Elute

Hybridize PNA probe for AD00370

Detect RNAi trigger guide strand by AEX/HPLC



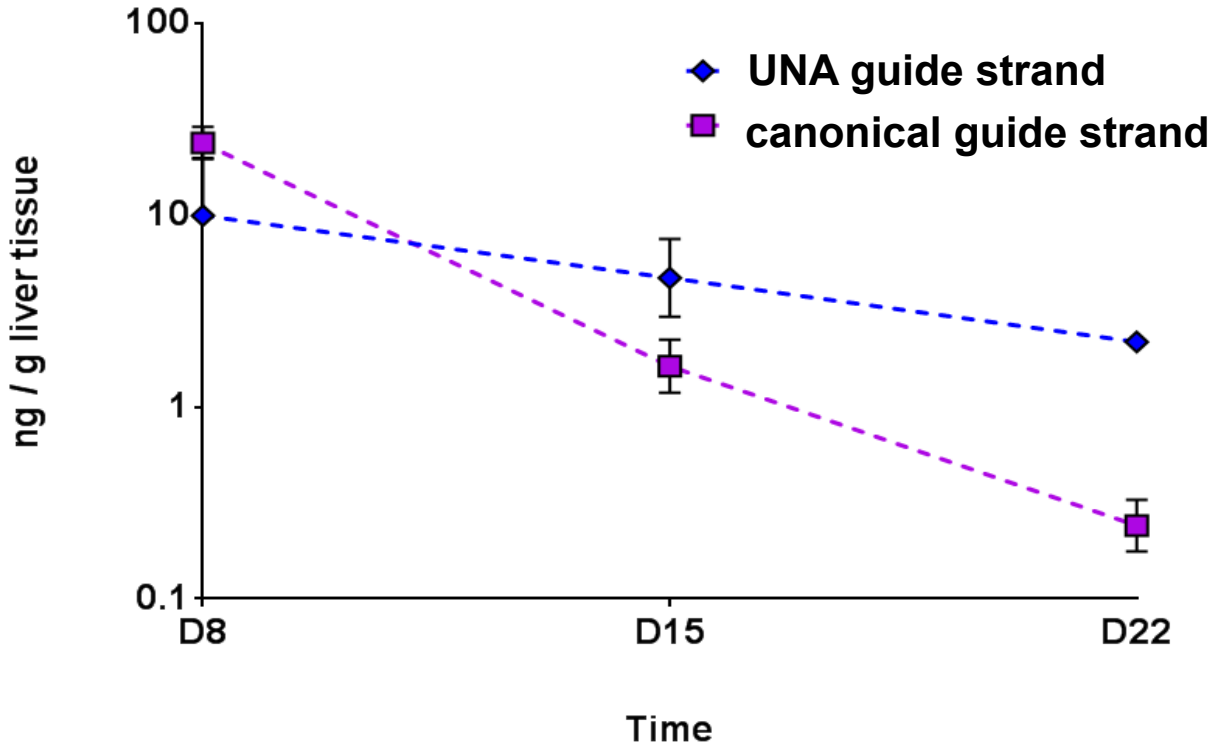


8 mg/kg AAT-UNA + 8 mg/kg ARC-EX1

Amount of UNA guide strand loaded in RISC is greater than canonical at later timepoints in liver

Ago2 pulldown assay

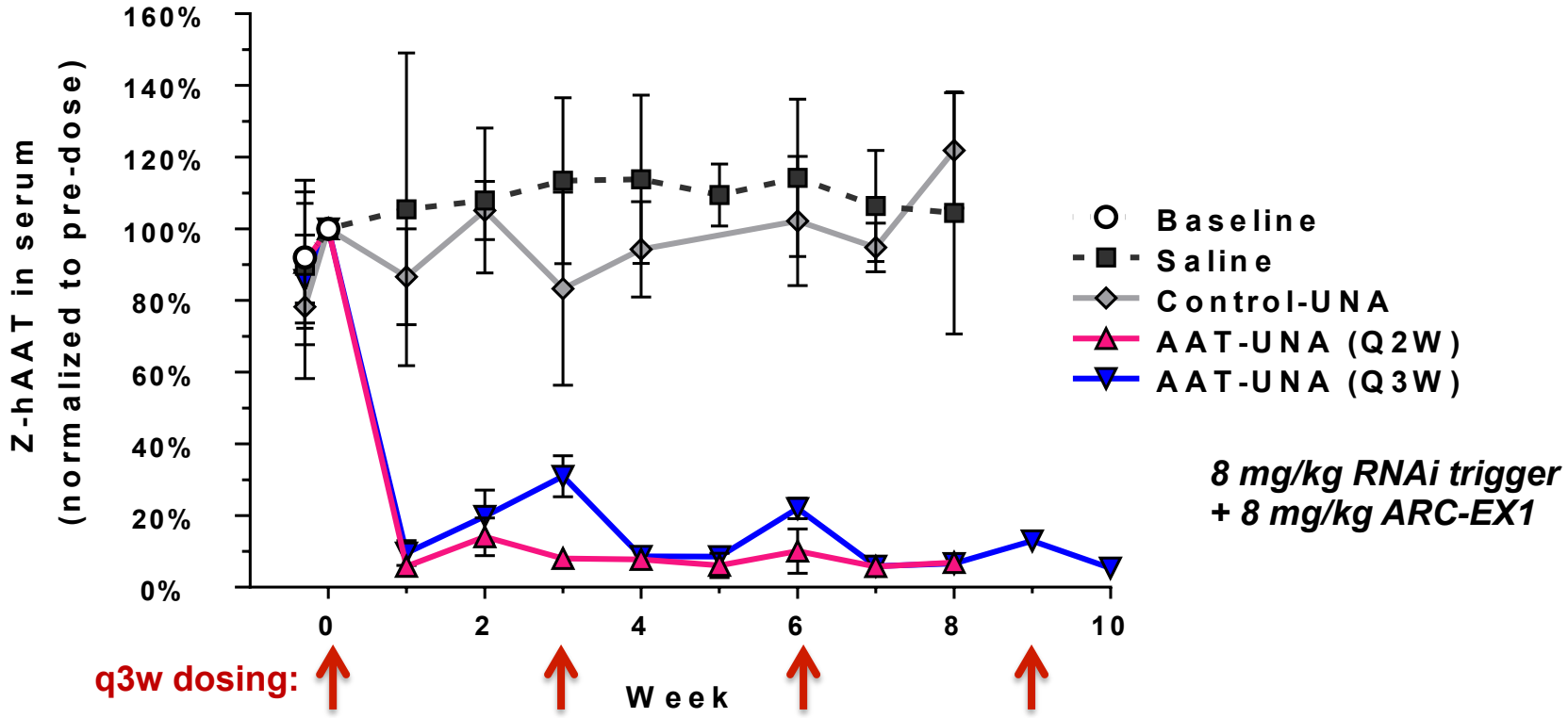
PiZ mouse liver lysate



Greater amount of RISC loaded with UNA guide strand at later timepoints is consistent with increased duration of knockdown.

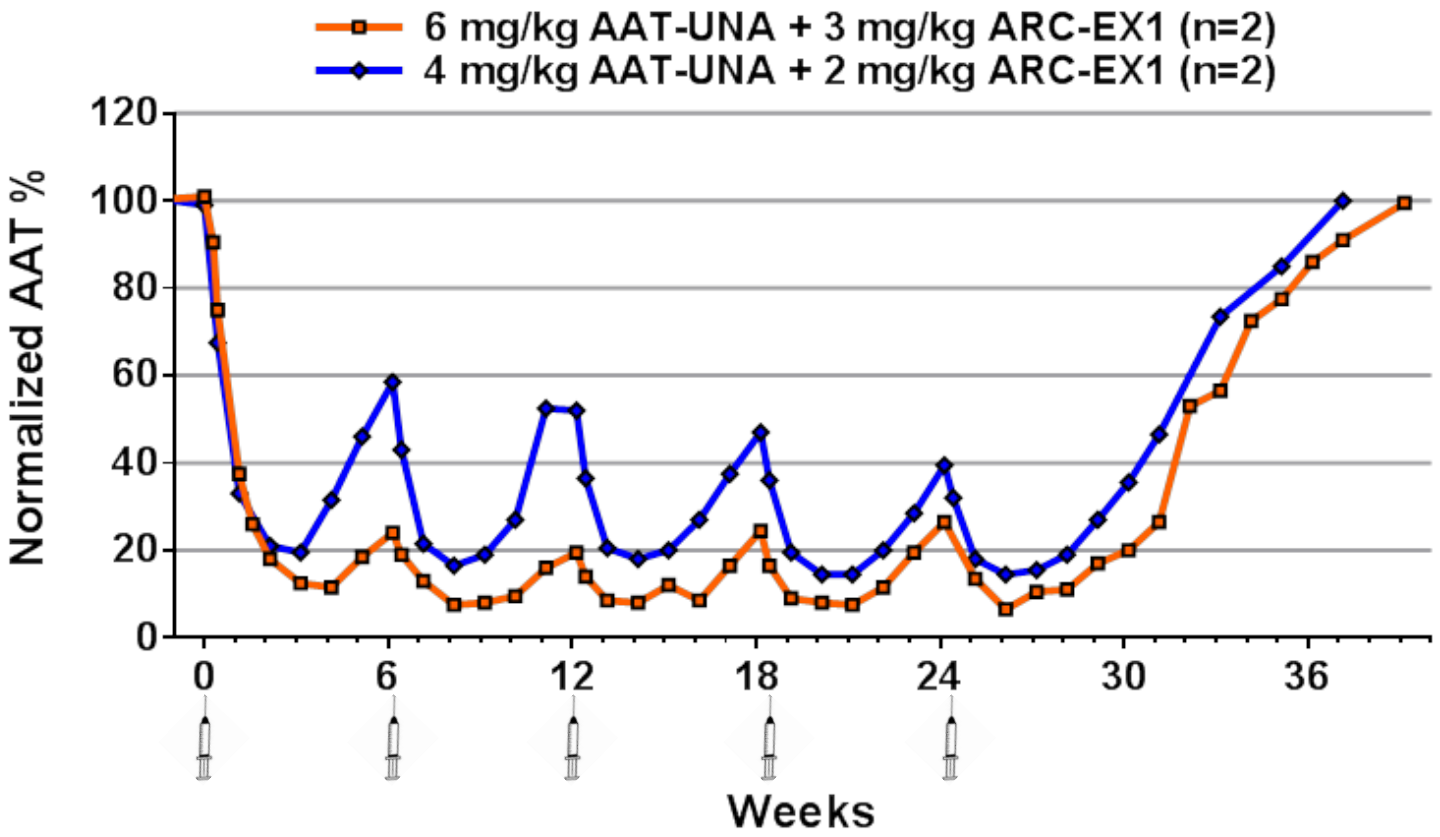
12 mg/kg RNAi trigger + 6 mg/kg ARC-EX1

Multiple dosing profile in PiZ mice



- Multiple dosing once every 2 or 3 weeks induces significant reduction in Z-hAAT.
- Cumulative effect upon repeat dosing.

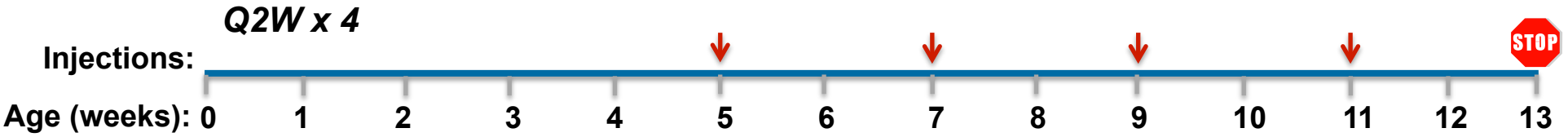
Long-term reduction of AAT in nonhuman primates following repeat dosing with ARC-AAT



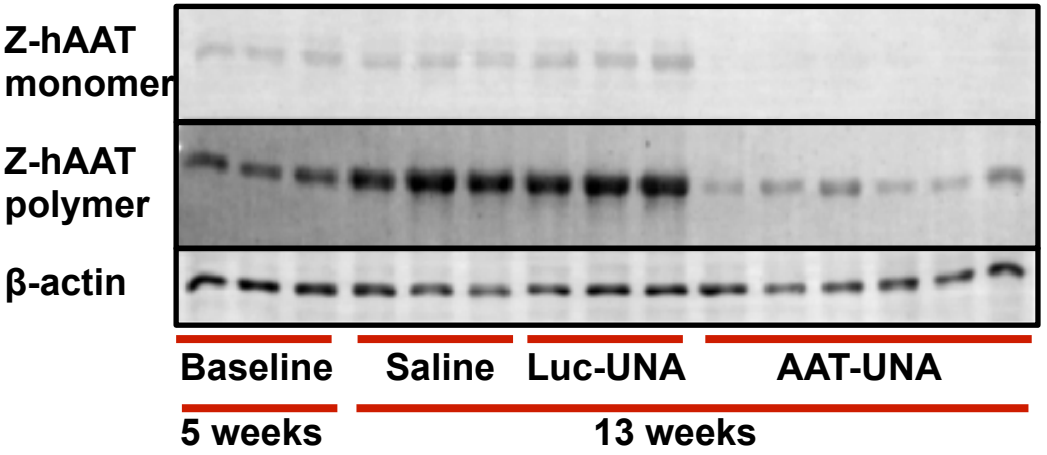
- Efficacious: ~90% reduction of serum AAT (6 mg/kg AAT-UNA)
- Sustained reduction of AAT with q6w dosing
- Cumulative effect of repeat dosing at 4 mg/kg AAT-UNA

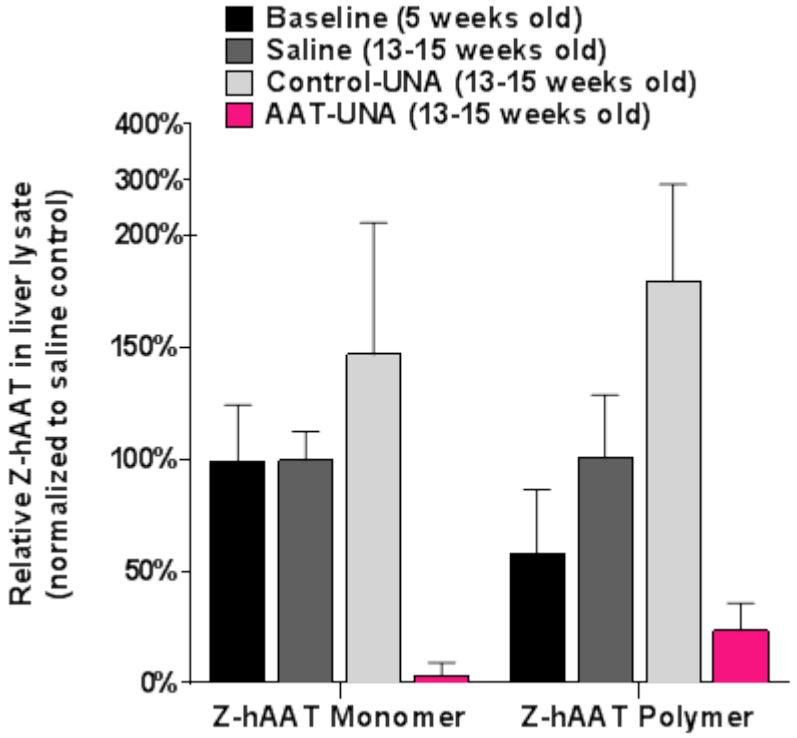
Reduction and prevention of Z-hAAT aggregates in liver

Young PiZ mice (males and females)



Western blot





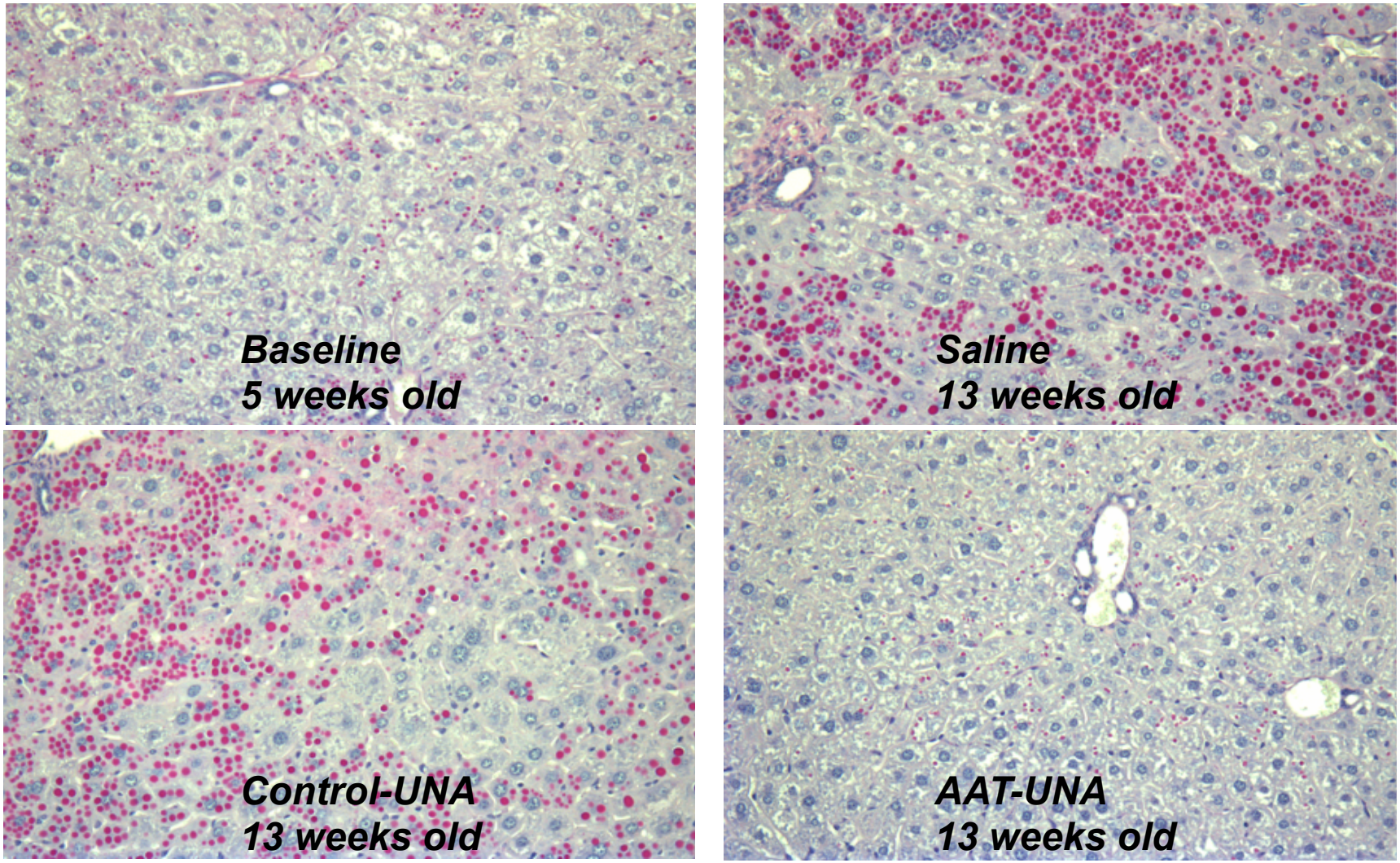
96% less soluble (monomer) Z-hAAT

76% less insoluble (polymer) Z-hAAT

8 mg/kg RNAi trigger + 8 mg/kg ARC-EX1

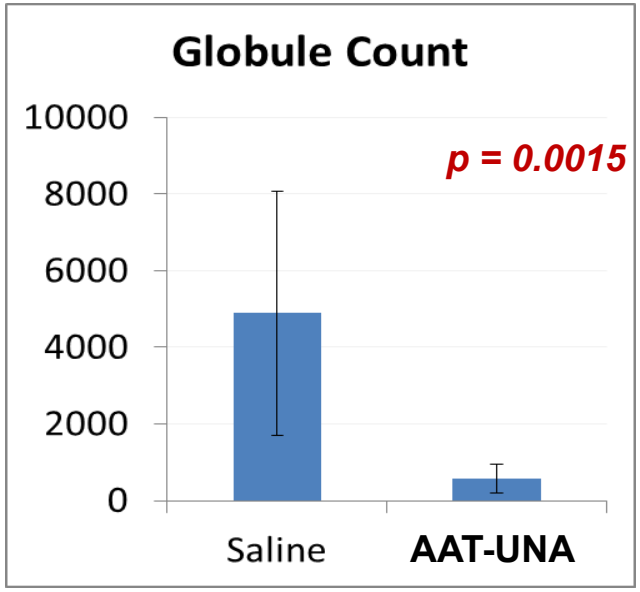
Prevention of Z-hAAT globules in PiZ mice

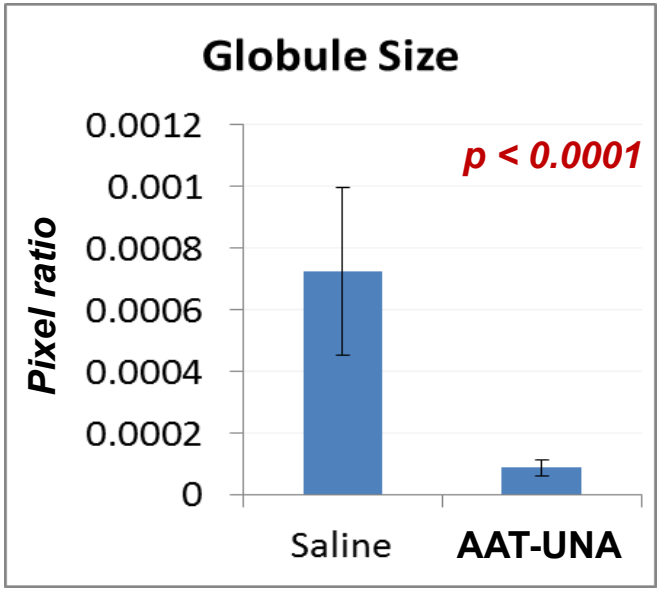
4 x q2w dosing

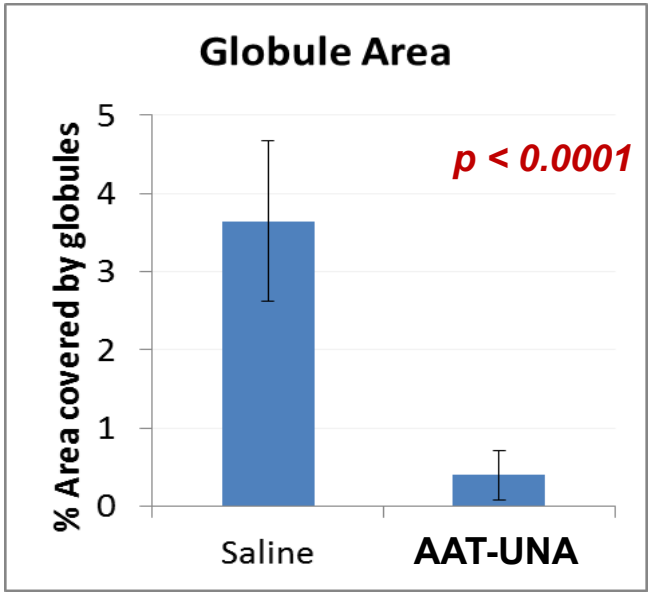


Male PiZ mice, 8 mg/kg RNAi trigger + 8 mg/kg ARC-EX1

Prevention of globule formation in young PiZ mice







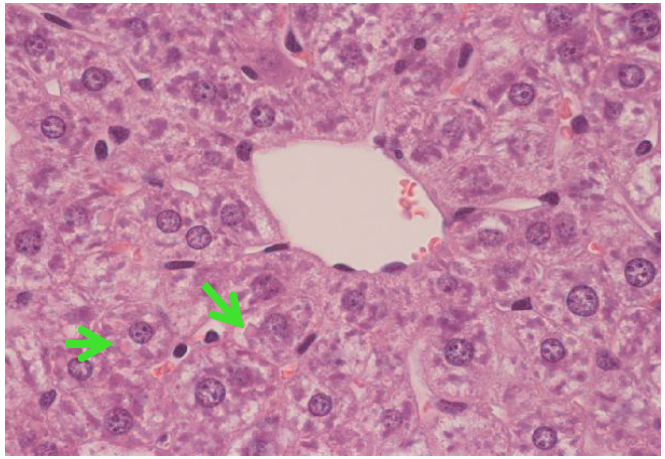
AAT-UNA treated mice had 85% fewer globules, 85% smaller globules, and 96% less area of the liver covered with globules than saline-injected controls.

8 mg/kg RNAi trigger + 8 mg/kg ARC-EX1

Prevention of Z-hAAT globule accumulation and inflammation in young PiZ mice

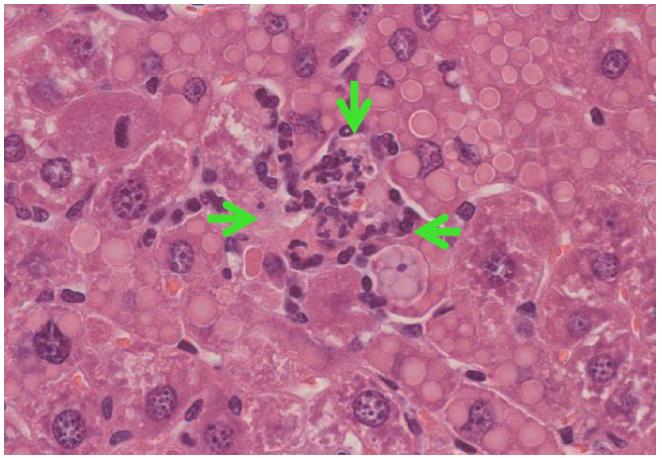
4 x q2w dosing

5 weeks old - baseline



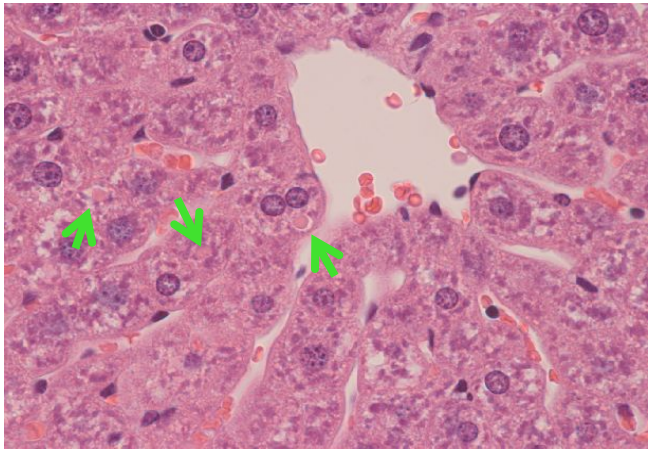
- Normal morphology
- Only a few, small Z-hAAT globules in hepatocytes (arrows)

13 weeks old (saline)



- Inflammatory infiltration around damaged or dead hepatocytes (arrows)
- Significant Z-hAAT globule accumulation in hepatocytes

13 weeks old (AAT-UNA)

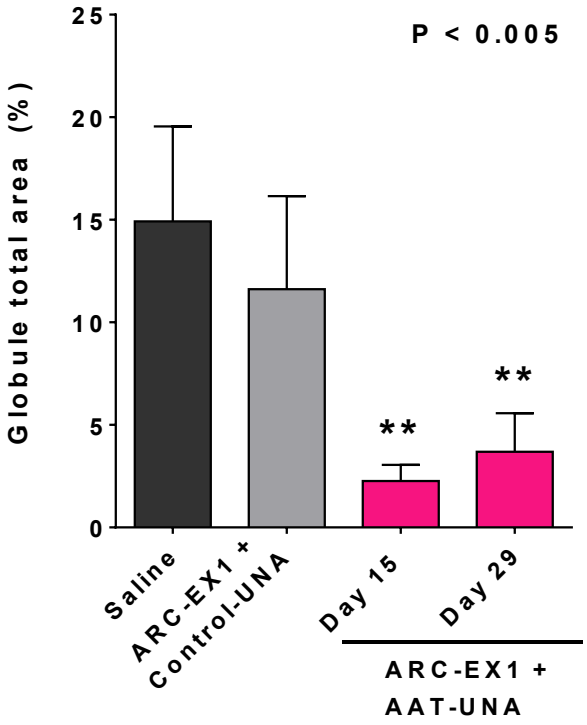


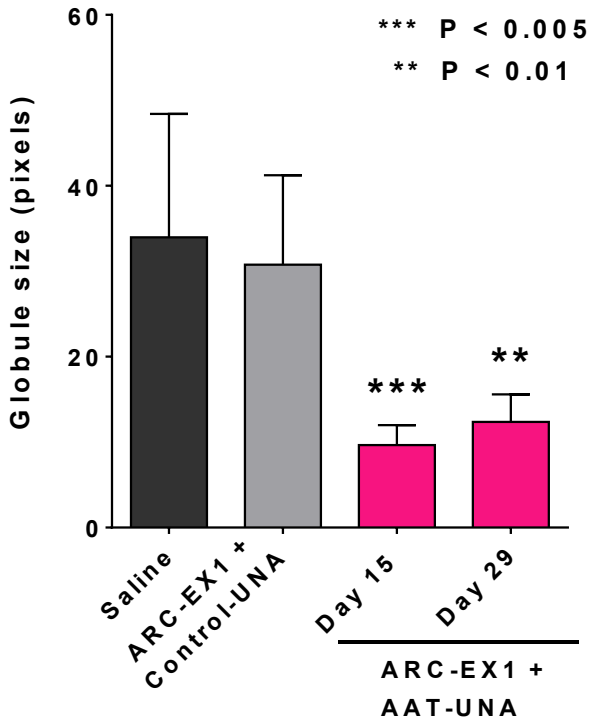
- Normal morphology, infiltrate absent
- Very rare, small Z-hAAT globules in hepatocytes (arrows)

Male PiZ mice
8 mg/kg RNAi trigger + 8 mg/kg ARC-EX1
H&E stained liver section, x1000

Reduction of pre-existing globules after a single ARC-AAT dose in older PiZ mice

6 month old PiZ mice
1 injection: 8 mg/kg RNAi trigger + 8 mg/kg ARC-EX1





Area covered by Z-hAAT globules and globule size within the liver decreased after a single injection.

ARC-AAT: Summary

- AATD is a large scale orphan disease affecting 100,000+ individuals in the US and a similar number in Europe.
- Improved lung outcomes with enzyme replacement therapy and resultant longer survival in adults is leading to a surge in recognized hepatic disease, and the emergence of HCC.
- AATD-associated liver disease has clinically severe manifestations in pediatric patients.

ARC-AAT: Summary

- AATD is a large scale orphan disease affecting 100,000+ individuals in the US and a similar number in Europe.
- Improved lung outcomes with enzyme replacement therapy and resultant longer survival in adults is leading to a surge in recognized hepatic disease, and the emergence of HCC.
- AATD-associated liver disease has clinically severe manifestations in pediatric patients.
- AATD-associated liver disease should be readily addressed by RNAi-based therapeutics.
- Injection of ARC-AAT in transgenic mice expressing human Z-hAAT results in prevention and reduction of Z-hAAT globules that lead to fibrosis and HCC.
- Injection of ARC-AAT in NHPs suggests once monthly or less frequent dosing is sufficient to maintain 80 - 90% knockdown.

ARC-AAT near-term clinical objectives

- ARC-AAT is intended to block PiZZ production in liver to reduce or eliminate liver injury associated with accumulation of PiZZ polymers
- Phase One, double-blind single-dose escalation now underway
  - Cohorts randomized 2:1 (active:placebo)
  - Part A: Cohorts enroll healthy normal volunteer subjects
  - Part B: Cohorts enroll PiZZ subjects
  - **Transition target: at least 30% reduction of serum AAT levels in 3 subjects or >60% reduction in a single subject, repeat dose level in PiZZ subjects (Part B)**
  - Continue dose escalation to maximal reduction in PiZZ plasma levels
  - Extensive safety evaluations and pharmacokinetic data

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