# ARO-AAT, a subcutaneous RNAi-based therapeutic for alpha-1 antitrypsin-related liver disease, demonstrates liver exposure-response and efficacy in preclinical studies

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# Disclosures

**C. Wooddell, H. Chen, J. Griffin, J. Hegge, D. Christianson, Z. Li, B. Given:** Employed by Arrowhead Pharmaceuticals

**J. Teckman:** Grant: Alnylam, Arrowhead Pharmaceuticals, Alpha-1 Foundation, Gilead, Consultant: Dicerna, Ionis Pharmaceuticals, Genkyotex, The Alpha-1 Project, RxCelerate, Editas, Intelia, AstraZeneca

**K. Blomenkamp:** None Declared

# Forward-looking Statements

This presentation contains forward-looking statements within the meaning of the "safe harbor" provisions of the Private Securities Litigation Reform Act of 1995. These statements are based upon our current expectations and speak only as of the date hereof. Our actual results may differ materially and adversely from those expressed in any forward-looking statements as a result of various factors and uncertainties, including, without limitation, the safety and efficacy of our product candidates, the duration and impact of regulatory delays in our clinical programs, our ability to finance operations, the timing for starting and completing clinical trials, rapid technological change in our markets, and the enforcement of our intellectual property rights. Our Annual Report on Form 10-K, recent and forthcoming Quarterly Reports on Form 10-Q, recent Current Reports on Forms 8-K, and other SEC filings discuss some of the important risk factors that may affect our ability to achieve the anticipated results, as well as our business, results of operations and financial condition. Readers are cautioned not to place undue reliance on these forward-looking statements. Additionally, Arrowhead disclaims any intent to update these forward-looking statements to reflect subsequent developments.

# 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

↓

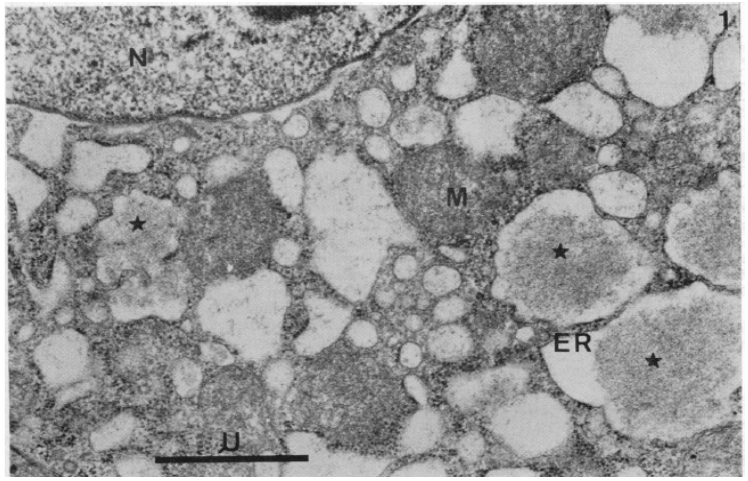
**Currently no treatment**

# ARO-AAT mechanism of action (RNA interference)

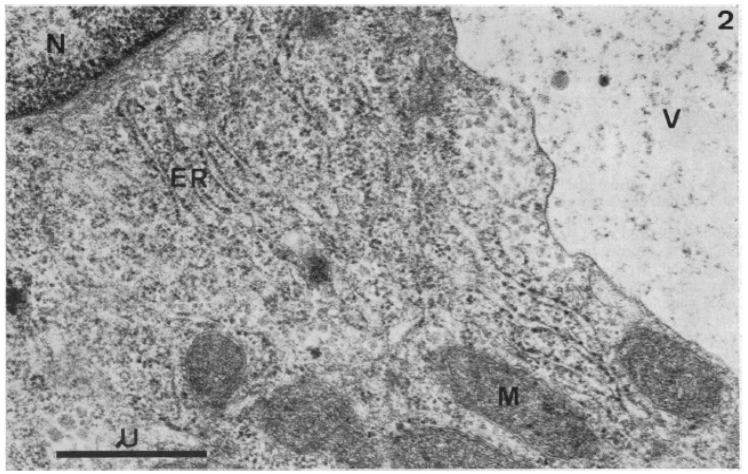
ARO-AAT designed to stop Z-AAT production by silencing AAT gene to:

- 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)**



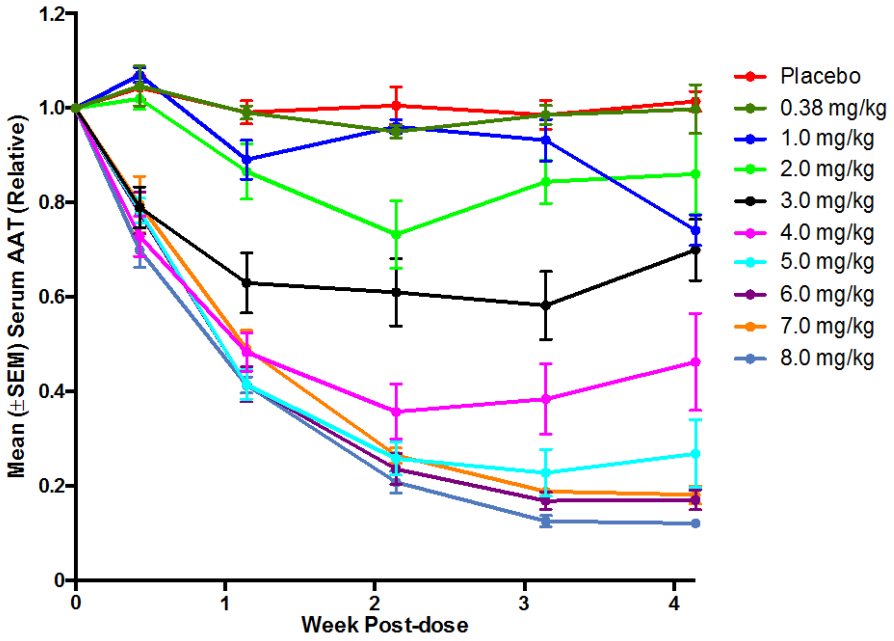

**Pi null phenotype (normal)**

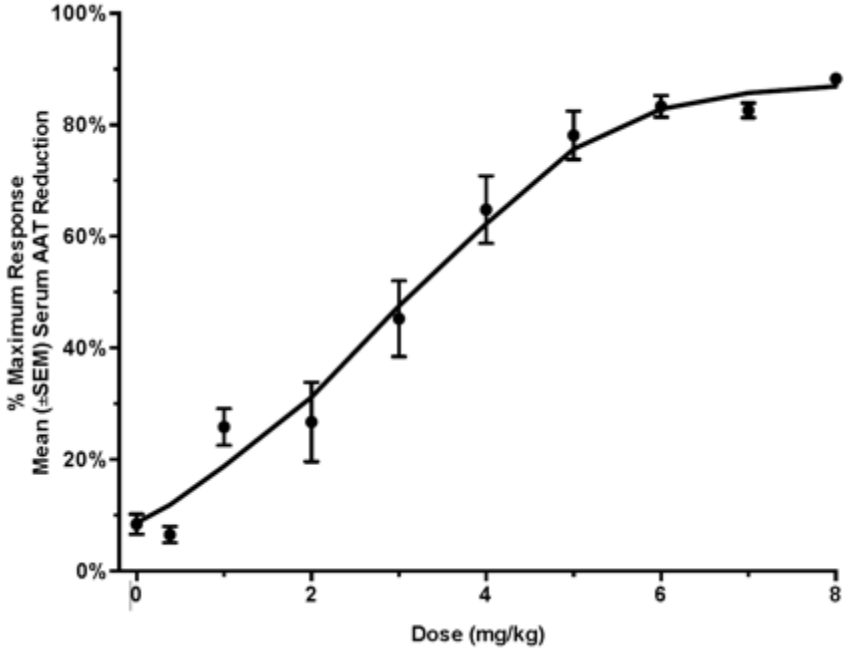



Feldmann G et al., *Gut* 1975

# Deep and durable AAT knockdown in healthy volunteers

Single-dose ARC-AAT (Arrowhead's discontinued 1st generation AAT RNAi therapeutic)




- Deep AAT knockdown (max. ~90%) with Arrowhead's first generation AAT RNAi drug consisting of RNAi trigger and endosome escape agent (EX1)
- Well tolerated in humans …but discontinued following findings due to endosome escape agent in animal toxicology study

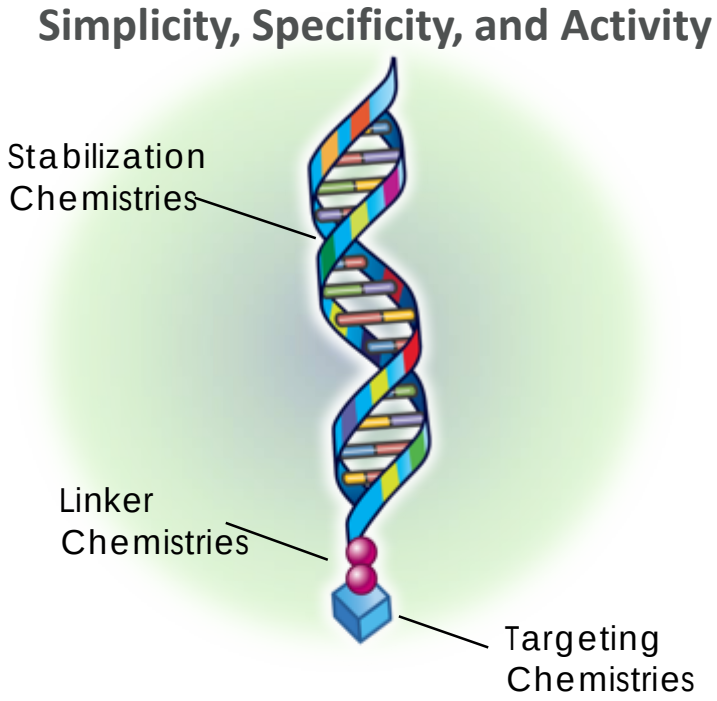
Turner et al (2018) J Hepatol online

# ARO-AAT: Key Design Elements Expected for the Next Generation

The Wish List:

- Subcutaneous dosing, monthly or less frequent
- No need for endosomal escape agent
- Full suppression of liver AAT production
  - Deep and prolonged knockdown of plasma AAT levels
- Expectation of wide therapeutic index
- Good tolerability in humans

# Arrowhead RNAi Platform: TRiM™




**TRiM™ has rules and algorithms to optimize trigger sequence**

- Limit cross reactivity with off target genes
- Maximize activity
- Maximize innate stability
- Rational use and placement of modifying chemistries

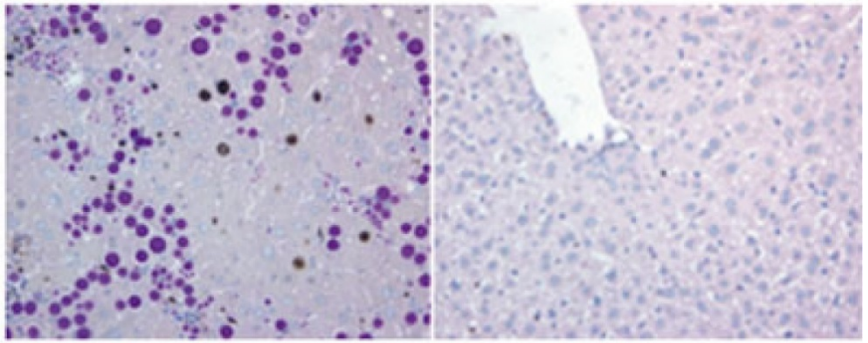
**Targeted RNAi Molecule**

**TRiM™ platform**

# PiZ mouse model of AAT deficiency liver disease

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

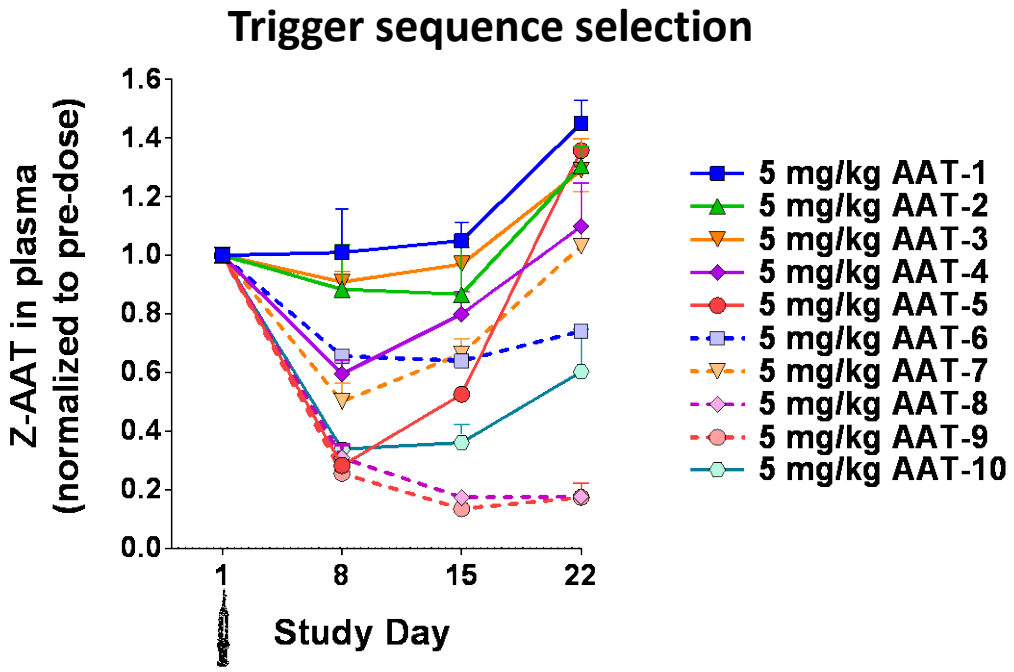
- Hepatocytes produce a large amount of human Z-AAT
- Hepatocytes are not able to efficiently process and secrete the Z-AAT
- Z-AAT forms polymers that accumulate in large "globules" within the hepatocytes
- Presence of polymer stresses hepatocytes, eventually leading to HCC
- Globules are visualized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

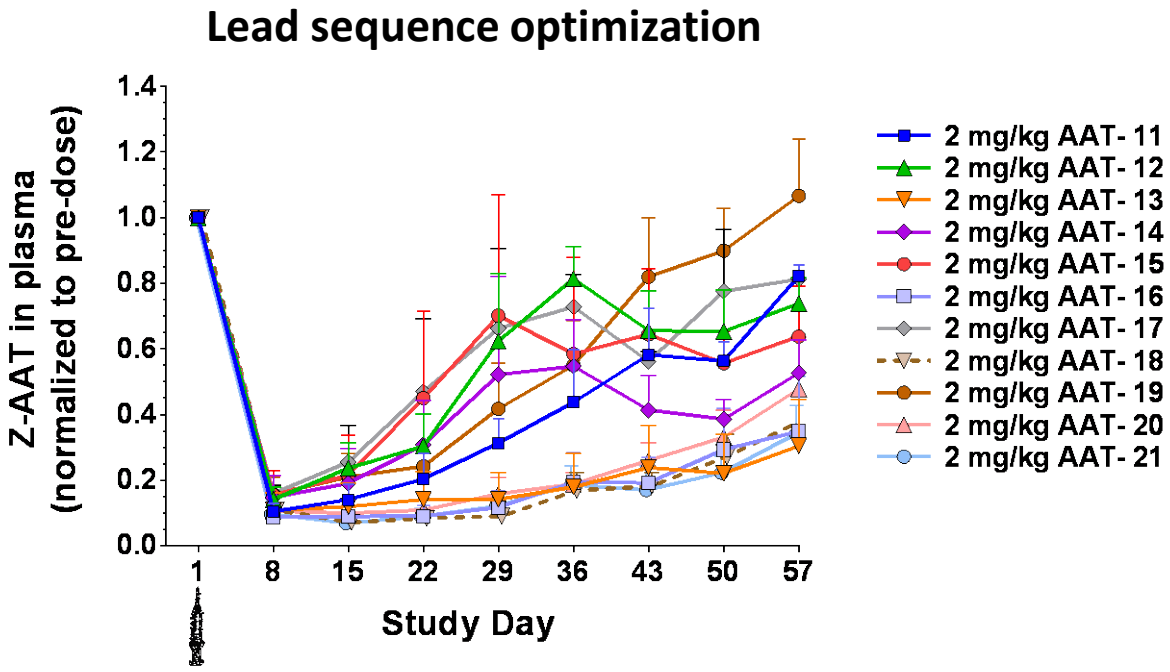


**PiZZ** **Wild Type**

Rudnick DA et al., *Hepatology*. 2004

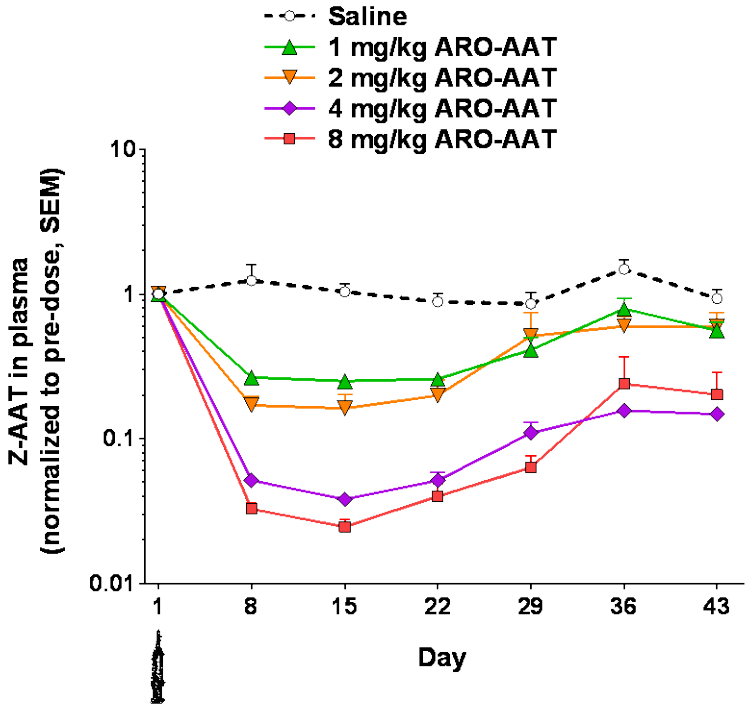
# RNAi trigger selection in PiZ mice

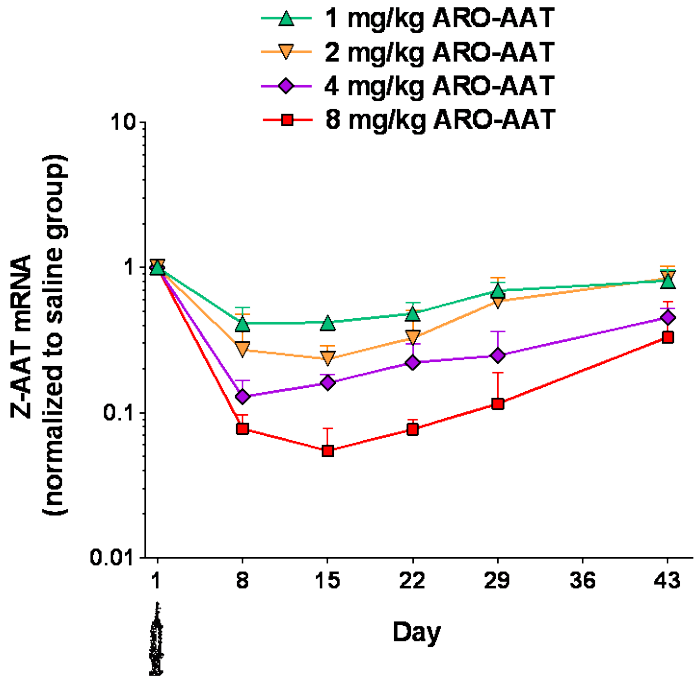


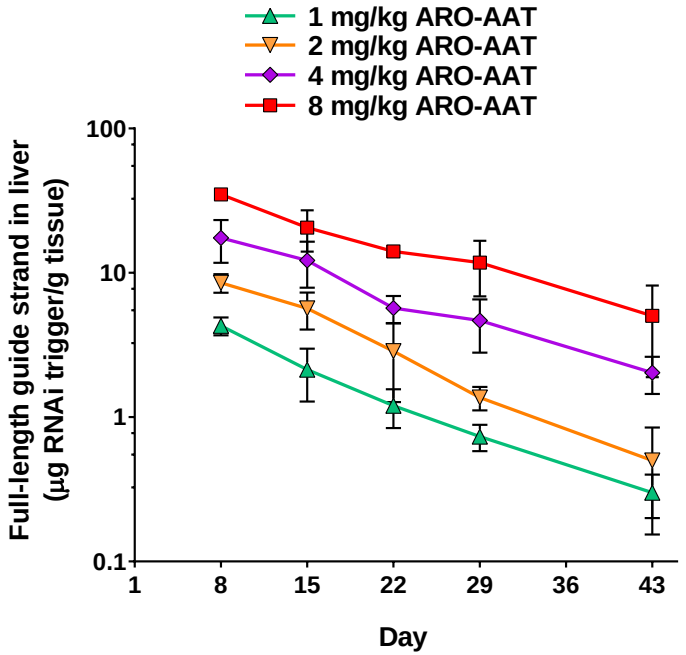




The most efficacious of the candidate sequences were optimized using the TRiM platform

# ARO-AAT dose response in PiZ mice



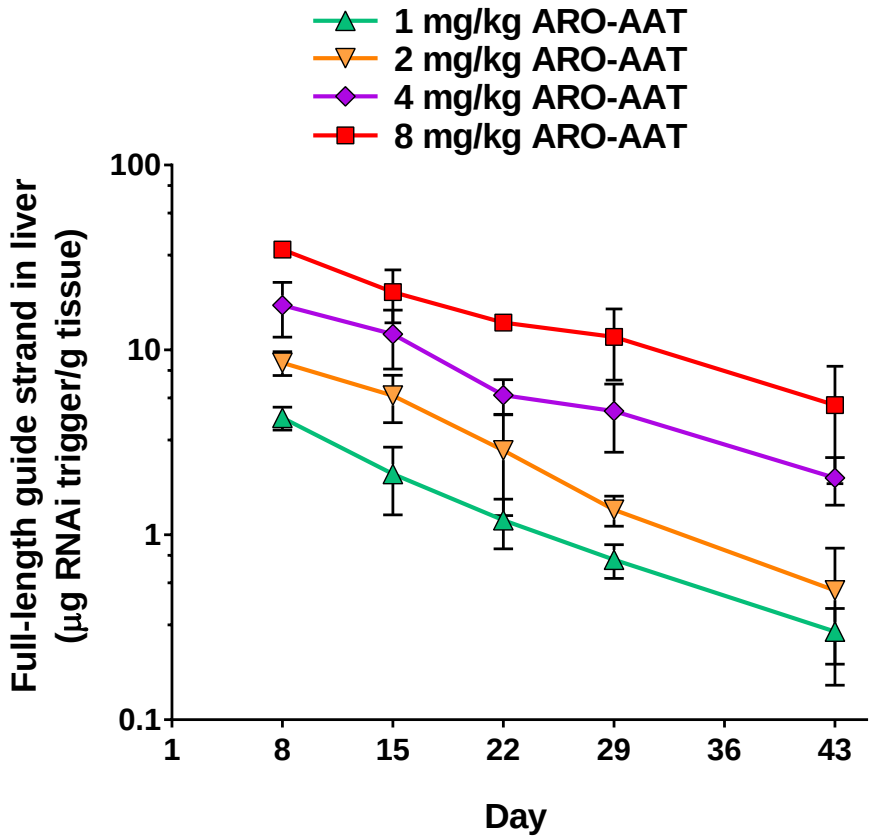



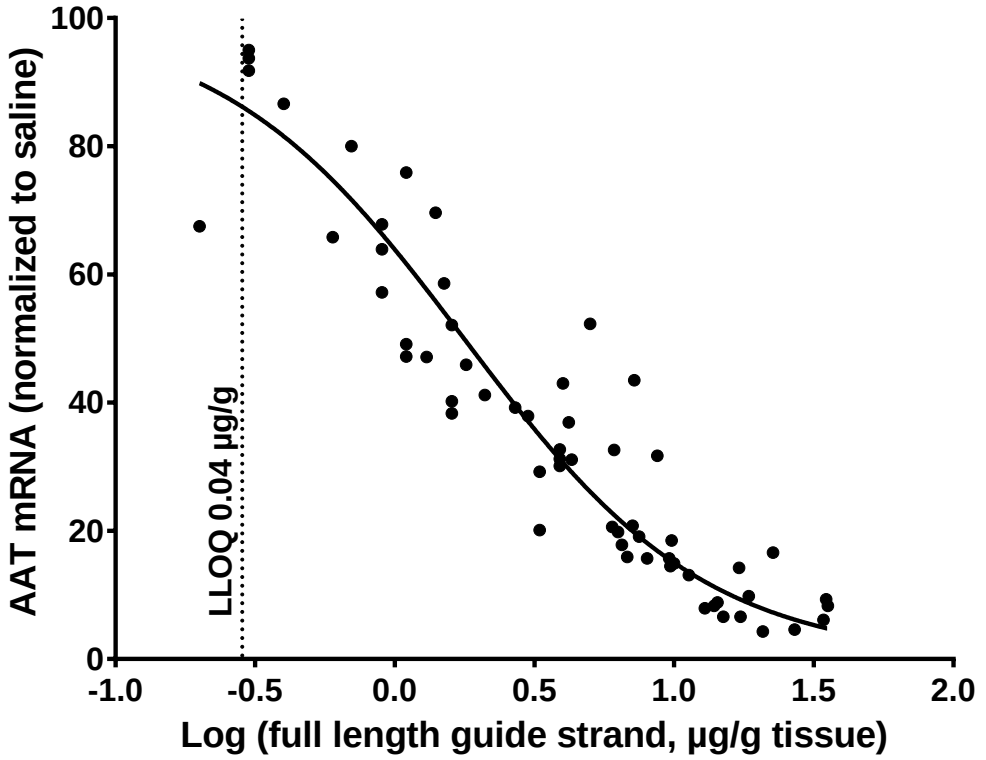

Dose and time dependent:

- Reduction of plasma Z-AAT protein and liver Z-AAT mRNA
- Amount of guide strand detected in liver tissue

RNAi trigger in liver tissue measured by chromatography using a hybridized fluorescent peptide-nucleic acid probe.

# Liver exposure-response relationship in PiZ mice




Z-AAT mRNA reduction correlates with amount of ARO-AAT guide strand in the liver

# Time course and biodistribution of ARO-AAT in rats

Single 3 mg/kg ARO-AAT subcutaneous injection in rats

| | µg RNAi trigger guide strand/ gram tissue | |
|---|---|---|
| **Time point** | **Liver** | **Kidney** |
| 1 hr | 19.9 | 1.8 |
| **4 hr** | **44.4** | **5.1** |
| 48 hr | 23.7 | 2.9 |
| 96 hr (4 days) | 16.8 | 3.5 |
| 192 (8 days) | 6.4 | 1.2 |
| 384 (16 days) | 1.4 | 0.5 |

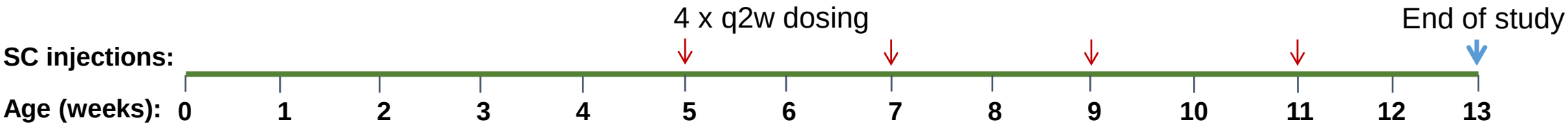
Plasma $t_{1/2}$ = 3.4 hr

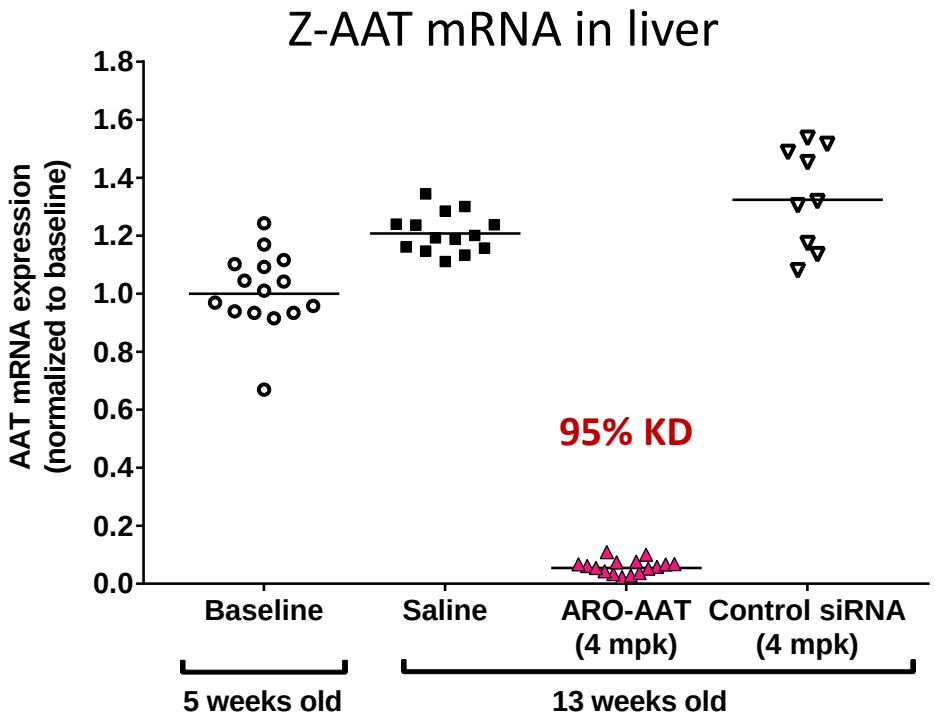
<u>At 4 hours:</u>

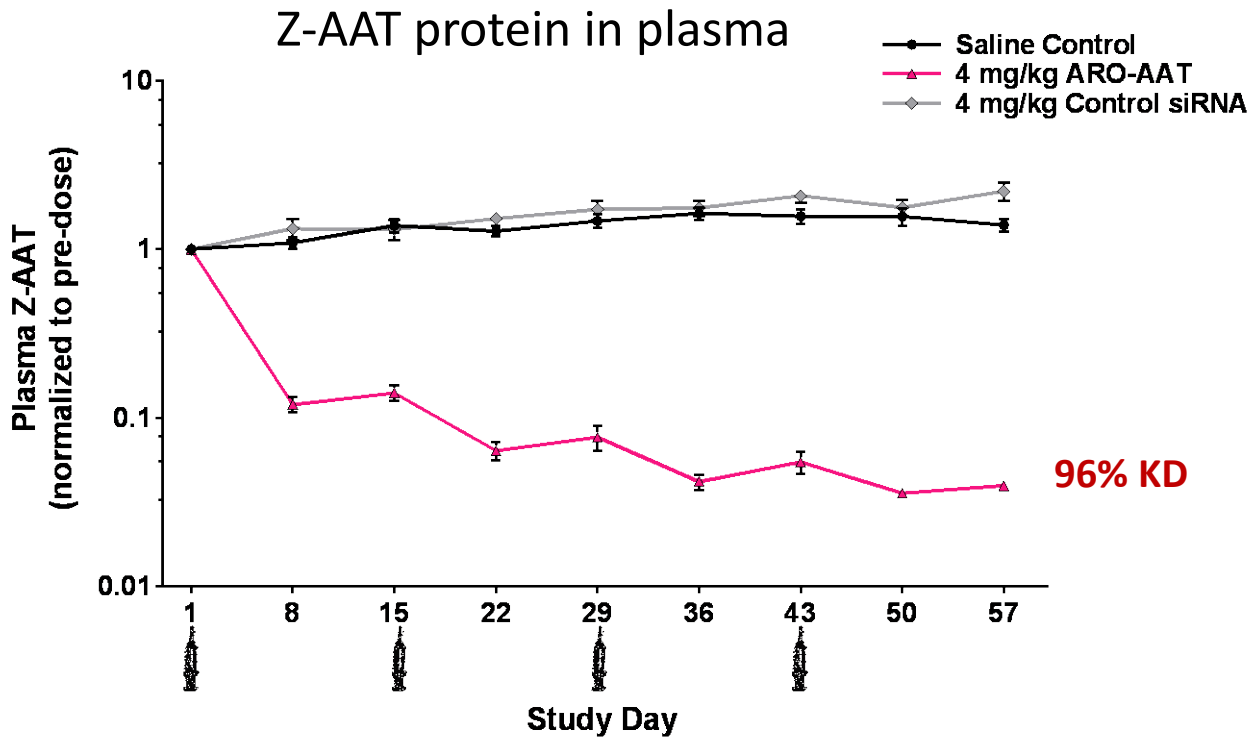
- Highest exposure in liver
- Exposure in kidneys was 8.7 fold lower
- No trigger detected in lung, brain, heart, spleen, adrenals

# Repeat dosing deeply reduced Z-AAT mRNA and plasma protein

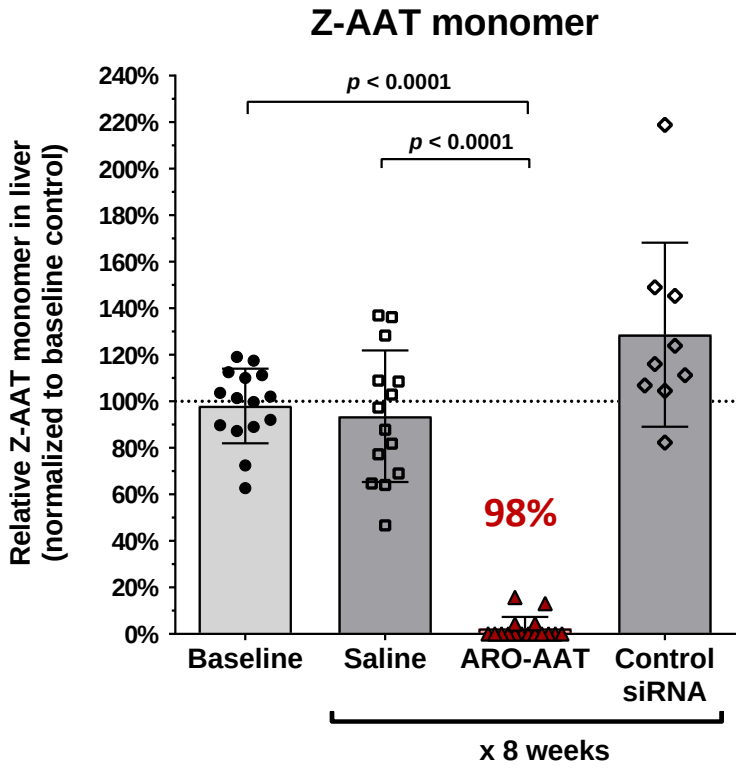
## PiZ mice

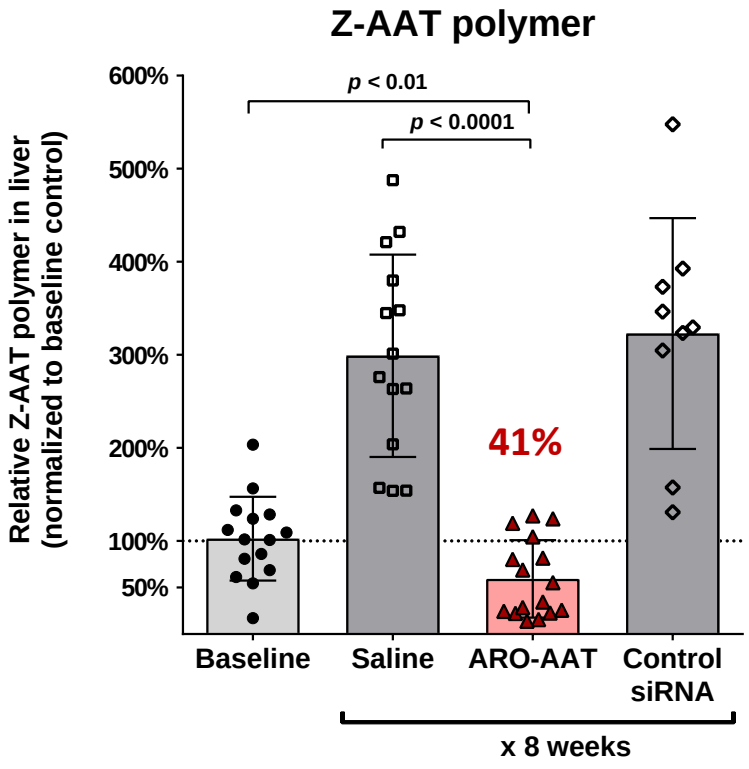


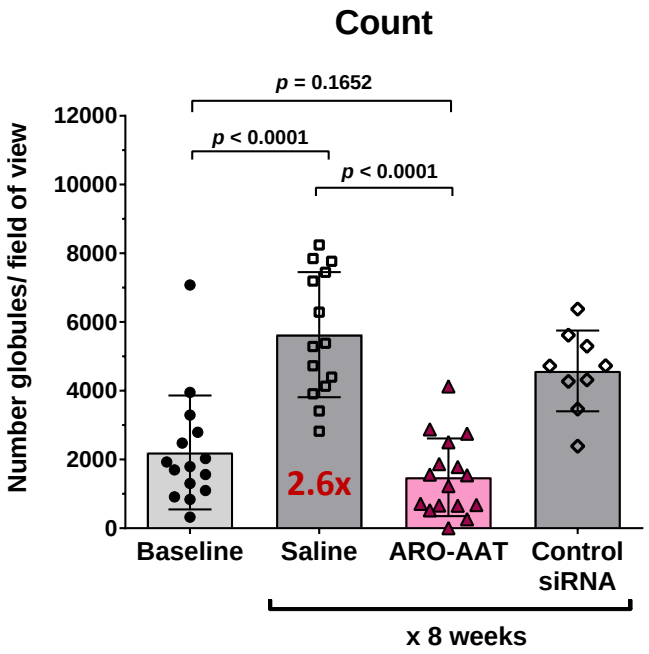
# Reduction of monomeric and polymeric Z-AAT protein in liver

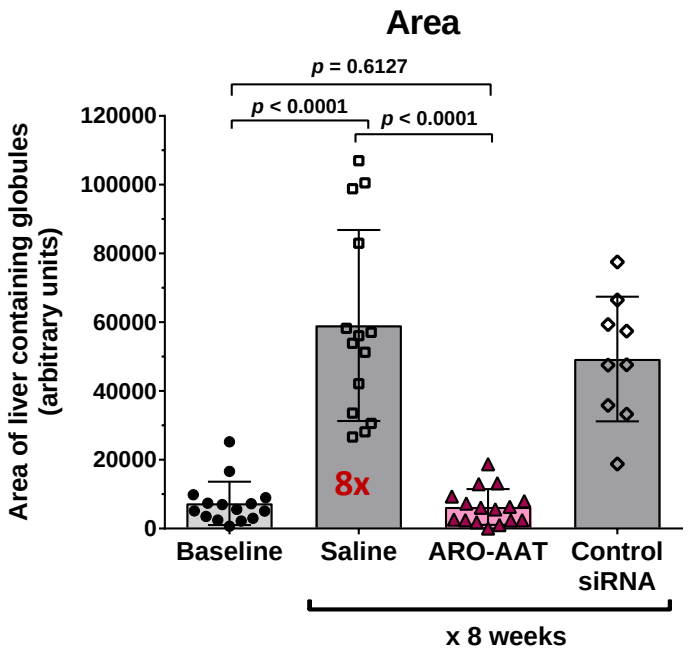


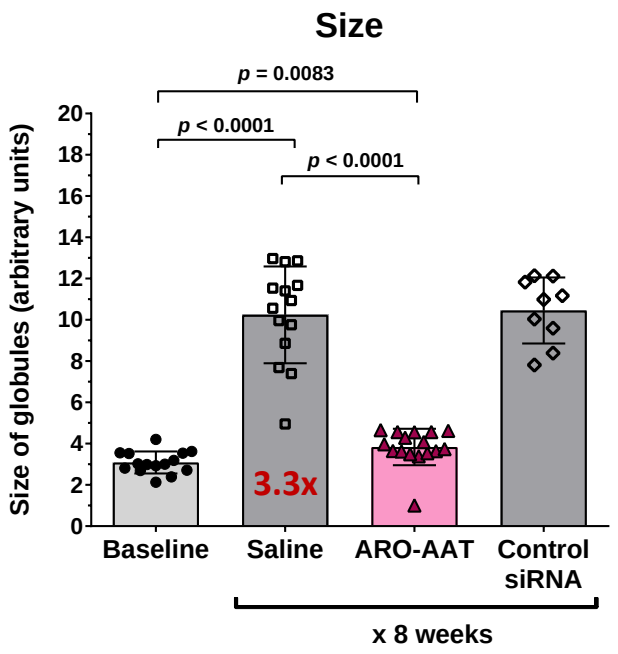



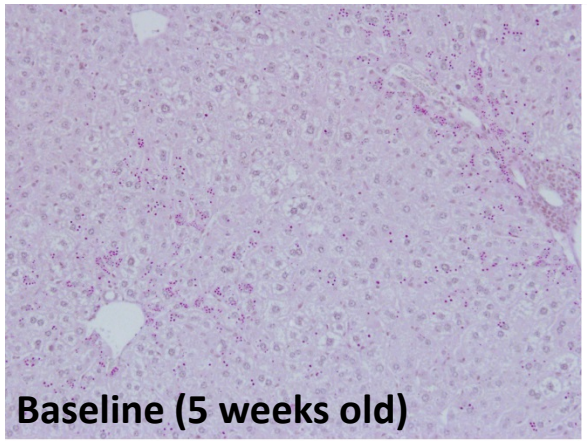

ARO-AAT treatment reduced soluble (monomeric) Z-AAT by 98% and insoluble (polymeric) Z-AAT by 41%, as well as preventing 3-fold polymer accumulation

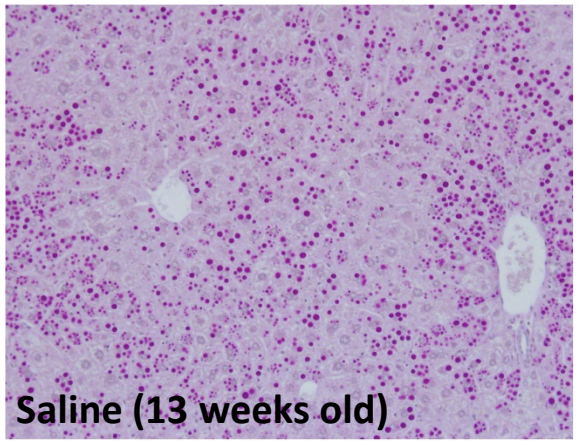
# Globule formation and accumulation prevented

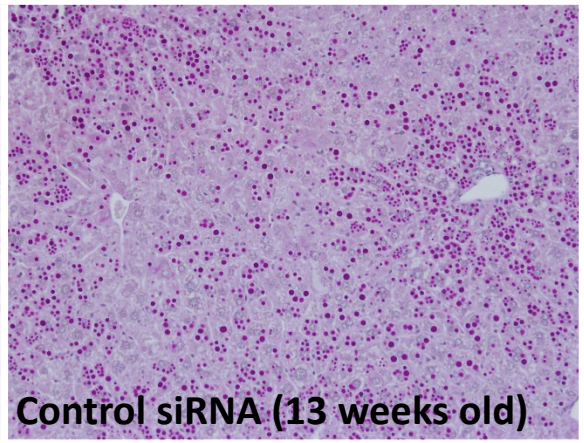


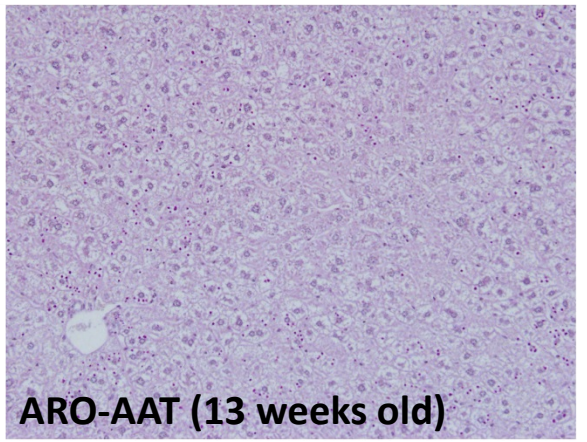



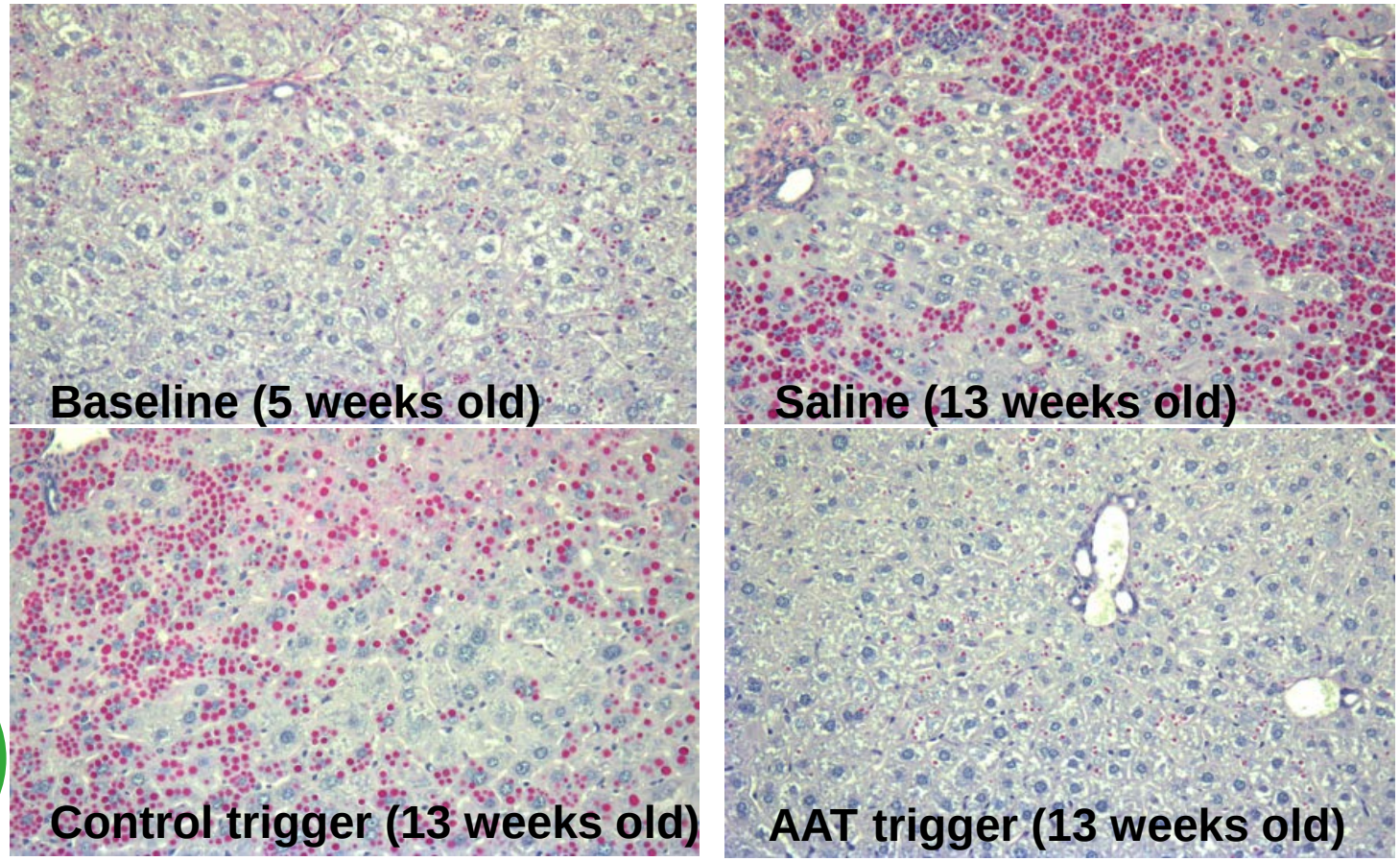




PAS-D stain

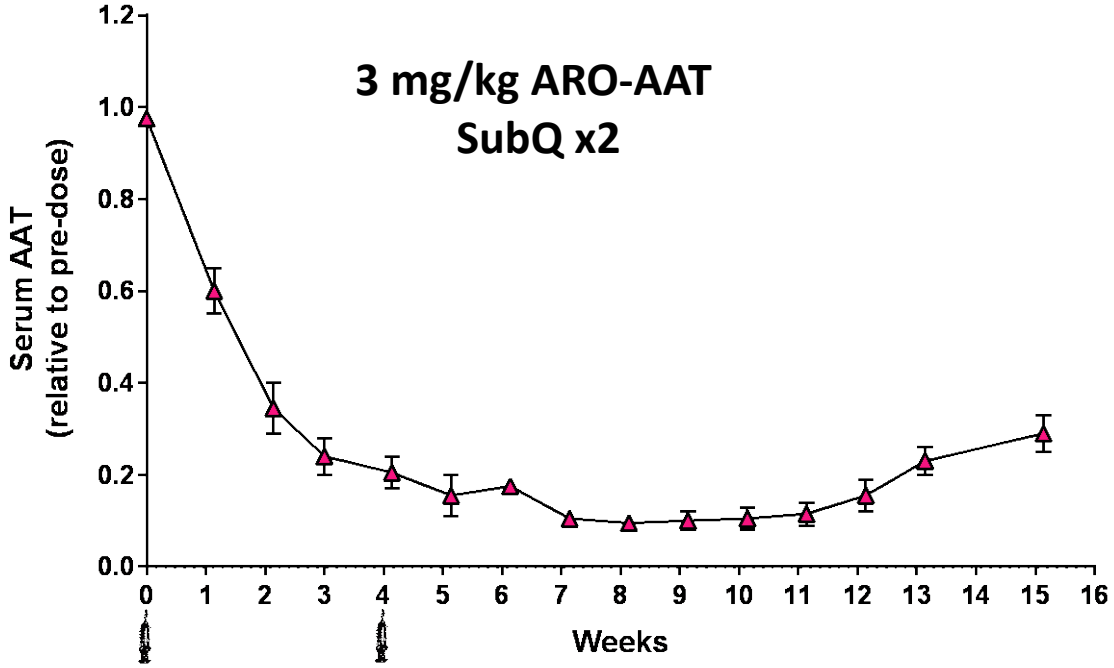
# Prevention of Z-AAT globules in young PiZ mice treated with components of 1st generation ARC-AAT

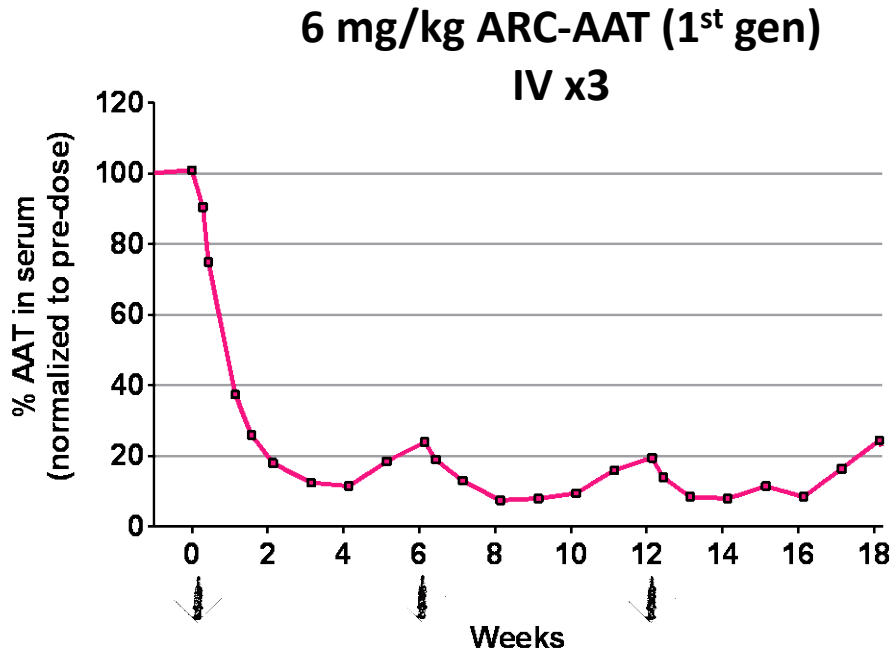
- 5-week old male PiZ mice
- Intravenous 4 x q2w dosing:
  - 8 mg/kg AAT trigger +
  - 8 mg/kg DPC (endosome escape agent)
- Result: Prevention of globule accumulation

Comparable results between 1st gen. ARC-AAT and ARO-AAT



# ARO-AAT provides durable AAT knockdown in NHPs




- Subcutaneous dosing, no endosome escape agent
- Mean 91% serum AAT KD with ARO-AAT
- KD sustained for 7+ weeks following second dose

- Intravenous dosing, limited safety margin
- 90% maximum serum AAT KD with ARC-AAT
- Rebound after 4 weeks

Durable knockdown from ARO-AAT supports once monthly or less frequent dosing

# In Conclusion…

- While known as a pulmonary disease and named for a circulating deficiency of alpha-1 antitrypsin, AATD is a storage disease in the liver
- While the liver disease has been known for decades and can be devastating in young children, its importance in adults is gaining appreciation with lower and later mortality from pulmonary disease
- Given learnings from other hepatic diseases where significant healing occurs with the withdrawal of injuring factors (such as virus), there is reason to hope that RNAi can stall the progression and possibly improve AATD-related liver disease
- ARO-AAT is a subcutaneously administered RNAi therapeutic with demonstrated efficacy:
  - Dose-dependent reduction of AAT mRNA in liver and AAT protein in plasma of PiZ mice
  - Prevention of disease-causing Z-AAT polymer production and reduction of pre-existing polymer
  - Deep and durable AAT knockdown in nonhuman primates
- **Clinical trial with ARO-AAT commenced March, 2018**

# Thank you !