Subcutaneous delivery of RNA interference (RNAi) therapeutic candidate for alpha-1 antitrypsin deficiency (AATD)-related liver disease produces deep and prolonged knockdown of plasma AAT

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Background

Alpha-1 antitrypsin (AAT) deficiency (AATD) is an autosomal co-dominant genetic liver disease. AAT is an abundant serum protein primarily synthesized in liver. Z-mutant AAT polymerizes and accumulates in hepatocytes, resulting in low serum levels, hepatocyte injury, and cirrhosis and hepatocellular carcinoma in some patients.

We previously reported on an intravenous RNAi-based therapeutic (ARC-AAT) that demonstrated proof of concept for RNAi efficacy in the PiZ mouse model that expresses human Z-AAT, and deep knockdown (KD) in healthy volunteers and patients (Wooddell et al, AASLD 2016, Turner et al, EASL 2016). KD of Z-AAT prevented and reversed accumulation of disease-causing Z-AAT polymers and inflammation, reduced expression of disease-related genes, and prevented tumors. However, a component of ARC-AAT that assisted in delivery of the RNAi trigger caused toxicity in nonhuman primates (NHPs) and ARC-AAT development was discontinued.

Here we describe a subcutaneous (subQ) therapeutic, ARO-AAT, producing deep and prolonged KD in PiZ mice and NHPs. We hypothesize that this inhibition of synthesis, and thereby elimination of the toxic Z-AAT accumulation in the liver, will prevent liver injury. ARO-AAT is expected to enter clinical development in 2018.

Methods

Key desirable attributes of an RNAi therapeutic to treat AATD liver disease

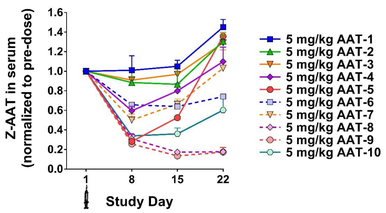
- ❑ SubQ dosing, monthly or less frequent
- ❑ Powerful AAT reduction
- ❑ Expectation of wide therapeutic index
- ❑ Efficacy and safety in AATD patients

Animal studies to demonstrate efficacy and safety

Efficacy of ARO-AAT was evaluated in the transgenic PiZ mouse model and in NHPs by measuring serum AAT using an ELISA kit for the mice (Abcam) and a Cobas Integra 400 Plus clinical chemistry analyzer for NHPs. Preliminary safety was assessed in rats and NHPs at dose levels up to and including 300 mg/kg, 100 times the expected human dose.

Results

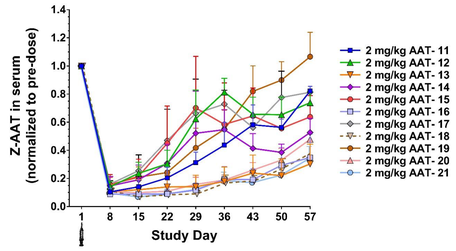
RNAi trigger sequence screening in PiZ mice



Candidate RNAi trigger sequences were screened for Z-AAT KD activity in female PiZ mice (48-84 weeks old) given a single subQ injection of 5 mg/kg RNAi trigger on Day 1. Plasma Z-AAT was measured by ELISA, shown with standard error (SEM).

The most efficacious of these RNAi trigger sequences was optimized by additional structure-activity relationship analysis.

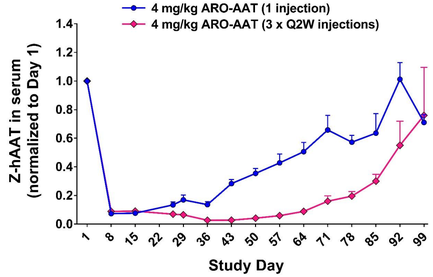
Optimization of RNAi trigger sequence in PiZ mice



Multiple RNAi triggers containing the same target site sequence but with different chemical structures were evaluated in male and female PiZ mice (19-29 weeks old) given a single subQ injection of 2 mg/kg RNAi trigger on Day 1. Plasma Z-AAT was measured by ELISA, shown with SEM.

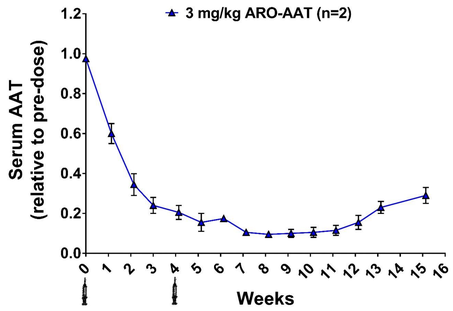
A subset of the most effective candidate triggers was further evaluated by single and repeat dosing in PiZ mice and NHPs. The selected RNAi trigger became drug product ARO-AAT.

Single and repeat dosing of ARO-AAT in PiZ mice



Female PiZ mice (14-24 weeks old) were given a single or 3 biweekly subQ injections (4 mg/kg) of RNAi trigger ARO-AAT. A mean maximum KD of 94% after a single dose and 97% following multiple doses was observed. Error shown as SEM.

Durable KD of serum AAT in nonhuman primates



Cynomolgus monkeys were given a subQ injection of 3 mg/kg ARO-AAT on Days 1 and 29.
Reduction in serum AAT of up to 92% in NHPs was observed following the two doses, with sustained KD 6-8 weeks after the second dose. Error shown as SEM.

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Rat safety study

Methods: 25 male Sprague-Dawley rats (Charles River Laboratories) were designated to 5 groups (5/group) and were dosed via subQ injection once weekly for three weeks at the dose levels of 0, 30, 60, 120, and 300 mg/kg ARO-AAT. Assessment of toxicity was based on clinical observations, hematology, clinical chemistry and coagulation. A necropsy was conducted on Day 16 and the lungs, kidney, liver, spleen, and adrenal glands were collected for histopathologic evaluation.

Clinical chemistry for liver function

Day	Parameter	Vehicle Control	Group 2 (30 mg/kg)	Group 3 (60 mg/kg)	Group 4 (120 mg/kg)	Group 5 (300 mg/kg)
2 (24 Hours Postdose)	Alanine aminotransferase (U/L)	55±11	50±8	58±6	49±4	54±8
	Aspartate aminotransferase (U/L)	74±8	70±6	77±10	63±6	65±8
	Alkaline phosphatase (U/L)	332±70	340±70	311±32	346±75	374±82

Day	Parameter	Vehicle Control	Group 2 (30 mg/kg)	Group 3 (60 mg/kg)	Group 4 (120 mg/kg)	Group 5 (300 mg/kg)
9 (24 Hours Postdose)	Alanine aminotransferase (U/L)	59±10	54±7	54±2	51±5	49±7
	Aspartate aminotransferase (U/L)	102±16	92±16	81±7	75±7	65±7
	Alkaline phosphatase (U/L)	339±60	307±61	294±46	336±84	343±83

Day	Parameter	Vehicle Control	Group 2 (30 mg/kg)	Group 3 (60 mg/kg)	Group 4 (120 mg/kg)	Group 5 (300 mg/kg)
16 (24 Hours Postdose)	Alanine aminotransferase (U/L)	51±10	48±5	48±4	48±7	42±7
	Aspartate aminotransferase (U/L)	63±9	91±26	78±9	75±36	69±16
	Alkaline phosphatase (U/L)	259±40	245±77	246±23	291±80	297±101

Test	Reference range in 9-16 week old male rats
Alanine aminotransferase	23 - 54 U/L
Aspartate aminotransferase	72 – 197 U/L
Alkaline phosphatase	61 – 217 U/L

Results: Clinical laboratory values were indistinguishable between test article and vehicle control groups.

Histopathology: None of the observed microscopic findings in rats were considered to be ARO-AAT related because they were singular in occurrence, were present at a comparable incidence in control rats and rats administered test article, and/or represent common, spontaneous, background findings in rats of this strain and age.

Pilot NHP safety study

Methods: 2 male cynomolgus monkeys were dosed 4 times at escalating dose levels of 30, 60, 120, and 300 mg/kg ARO-AAT with one week between each dose. Clinical chemistry and hematology samples were collected pre-dose and the day after each dose (~24 hr post-dose). Animals were necropsied on Day 23 after the last dose.

Results: No abnormal clinical observations, body weight changes, clinical chemistries nor organ weight findings were noted. Hematology had slight decreases in red cell mass parameters and an increase in reticulocytes, but was likely secondary to the bleeding schedule, and was not considered adverse. Histopathology findings were limited to edema at the injection sites for each monkey, but were considered non-adverse.

Conclusions

ARO-AAT, a new subcutaneous therapeutic candidate, provides deep and durable KD in PiZ mice and NHPs. Based on clinical observations, clinical chemistries and limited histopathology evaluations, ARO-AAT was well tolerated at doses up to and including 300 mg/kg in rats and NHPs. This RNAi therapeutic holds promise for monthly or less frequent treatment of patients with AATD-associated liver disease.