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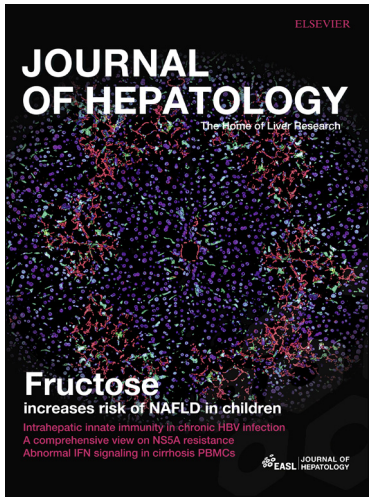

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Hepatic-targeted RNA interference provides persistent knockdown of alpha-1 antitrypsin levels in ZZ patients

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Abstract

Background & Aims: Alpha-1 antitrypsin deficiency (AATD) is a genetic disorder causing pulmonary and liver disease. The PiZ mutation results in mis-folded alpha-1 antitrypsin protein (Z-AAT) leading to hepatocyte accumulation, fibrosis and cirrhosis. RNAi-based therapeutics silencing production of hepatic Z-AAT might benefit patients with AATD-associated liver disease. This study evaluated an RNAi therapeutic to silence production of alpha-1 antitrypsin.

Methods: Part A of this double-blind first-in-human study randomized 54 healthy volunteers (HVs) into single dose cohorts (2 placebo: 4 active), receiving escalating doses of the investigational agent ARC-AAT from 0.38 to 8.0 mg/kg or placebo. Part B randomized 11 PiZZ genotype AATD patients who received up to 4.0 mg/kg or placebo. Patients with baseline FibroScan® >11 kPa or FEV_1 (forced expiratory volume in one second) < 60% were excluded. Assessments included safety, pharmacokinetics, and change in serum alpha-1 antitrypsin (AAT) concentrations.

Results: 36 healthy volunteers received ARC-AAT and 18 received placebo (Part A). Seven PiZZ individuals received ARC-AAT and 4 received placebo (Part B). A dose-response in serum AAT reduction was observed at doses ≥ 4 mg/kg with similar relative reductions in PiZZ patients and HVs at 4 mg/kg and a maximum reduction of 76.1% (HVs) vs. 78.8% (PiZZ) at this dose. Time for serum AAT return to baseline was similar for HV and PiZZ. There were no notable differences between HV and PiZZ safety parameters. The study was terminated early due to toxicity findings related to the delivery vehicle (ARC-EX1) seen in a non-human primate study.

Conclusion: PiZZ and HV responded similarly to ARC-AAT. Deep and durable knockdown of hepatic AAT production based on observed reduction in serum AAT concentrations was demonstrated.

Lay Summary: Accumulation of abnormal proteins in the livers of patients with alpha-1 antitrypsin deficiency may lead to decreased liver function and potentially liver failure. Therapeutics targeting the production of these abnormal proteins may be used to prevent or treat liver disease in patients with alpha-1 antitrypsin deficiency.

List of Abbreviations: AATD, alpha-1 antitrypsin deficiency; Z-AAT, mutant mis-folded protein expressed by Z-allele; HVs, healthy volunteer; ARC-AAT, RNA therapeutic targeting alpha-1 antitrypsin; FEV1, forced expiratory volume in one second; AAT, alpha-1 antitrypsin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ARC-EX1, excipient used in ARC-AAT to facilitate siRNA delivery; RNAi, ribonucleic acid interference; BMI, body mass index; ER, endoplasmic reticulum; KD, knockdown; siRNA, short interfering ribonucleic acid; PK, pharmacokinetics; PBO, placebo; DLT, dose limiting toxicity; AE, adverse event; ECG, electrocardiogram; VC, vital capacity; DLCO, diffusing capacity of the lungs for carbon monoxide; ATS, American Thoracic Society; ERS, European Respiratory Society; SAE, serious adverse event; API, active pharmaceutical ingredient; LC/MS/MS, liquid chromatography-mass spectrometry; API-AAT, RNA component of ARC-AAT; chol-UNA, cholesterol-conjugated unlocked nucleobase analog; mRNA, messenger RNA; SOC, system organ class; MLP, mellitin like peptide; Cmax, maximum serum concentraction; Tmax, time to reach Cmax; AUC0-24, area under the curve from 0-24 hours; AUCinf, area under the curve time 0 to infinity; t1/2, half-life; PD, pharmacodynamic; ELF, enhanced liver fibrosis panel

Introduction

Alpha-1 antitrypsin deficiency (AATD) is an autosomal co-dominant disease which predisposes to early-onset emphysema and liver cirrhosis [1]. Estimates of the number of adult AATD patients exhibiting clinically significant liver disease have varied from 10% (defined by transaminase abnormalities) [2, 3] to 18% in a U.K. cohort assessed by ultrasound (+/- biopsy) [4] to approximately 35% in those with an Ishak fibrosis score ≥ 2 assessed histologically after liver biopsy [5]. A Swedish autopsy series from AATD patients with the PiZZ genotype found liver disease to be present in the majority of patients, albeit unrecognized in many cases during life [6]. In common with other causes of cirrhosis, many factors are thought to affect clinical presentation, including alcohol use [7], BMI [8], gender [9] and genetic modifiers. Liver disease shows a bimodal clinical presentation. Prolonged neonatal jaundice is common in newborns with the PiZZ genotype, with most recovering but others progressing to cirrhotic disease that requires transplantation at a young age [10, 11]. A second peak occurs in adults who can present with clinical liver disease that can also progress to cirrhosis and transplant, even in the absence of a childhood history of liver disease [3]. No definitive methods to predict progression to cirrhosis exist at present in either the pediatric or adult populations. Current management of liver disease in AATD is largely supportive, with transplantation the only definitive treatment. In the U.S., AATD accounts for approximately 1.5% of liver transplants with ~76 transplants performed annually [12] with outcomes similar to other common indications for transplant [13].

The most common genetic variant of alpha-1 antitrypsin (AAT) associated with liver disease is the PiZ allele whose protein product misfolds and forms polymers that are retained within the endoplasmic reticulum (ER) of hepatocytes and can be seen as periodic acid-Schiff-positive inclusions in biopsies of AATD livers [14]. Drugs targeting polymerization,

as well as those that influence cellular homeostasis mechanisms which target disposal of the mutant protein aggregates (such as autophagy) represent potential novel therapeutic routes [14]. Another route to reduce AAT polymers would be to limit production of alpha-1 antitrypsin protein by interfering with its mRNA using antisense or RNA interference target knockdown (KD) approaches. This KD method has demonstrated reduced accumulation of mutant protein globules and inhibited expression of fibrosis-associated genes in the PiZZ transgenic mouse [15]. We report here results of a randomized, placebo-controlled phase 1 study in healthy volunteers (HVs) and PiZZ AATD individuals administered ARC-AAT, a short interfering RNA (siRNA) therapeutic targeting liver production of AAT. Preclinical animal studies with ARC-AAT will be reported elsewhere.

Materials and Methods

This was a multi-center, randomized, placebo-controlled, double-blind, single dose-escalation, first-in-human, Phase 1 study (NCT02363946) conducted to evaluate the safety, tolerability, pharmacokinetics (PK) and effect on circulating AAT levels of the investigational product, ARC-AAT, administered intravenously to healthy volunteers (Part A) and to PiZZ patients with AATD (Part B). Part A was initiated in HVs and Part B in individuals with AATD. With the intention of not enrolling rare disease patients at doses unlikely to have activity, Part B proceeded once target serum AAT KD of at least 30% in three of six HVs in a Part A cohort was seen, together with satisfactory safety data. The design is summarized in Fig. S1, S2. The study was approved by the appropriate institutional review committee at each site prior to commencement. Written informed consent was obtained from each subject included in the study and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by

each institution's human research committee. Participants who met all eligibility criteria (see Supplementary methods) were randomized using block randomization at a ratio of 2:1 (active:placebo) to receive a single intravenous injection of either placebo (PBO) or ARC-AAT in double-blind fashion. The study allocation sequence was generated and patients were assigned to study groups by an un-blinded statistician at the contract research organization (CPR Pharma, Adelaide, Australia). The study investigator at each site enrolled all study participants. Once screened, eligible participants were allocated to a sequentially numbered treatment and assigned a unique randomization number in accordance with the randomization schedule. The study protocol specified that all study participants and investigators remained blinded to treatment group throughout the study. The sponsor was permitted to unblind on a cohort by cohort basis after all subjects in a cohort completed their final study visit. The first study participant was enrolled December 5th, 2014 and the last participant follow up visit was November 18th, 2016.

Up to thirteen cohorts (6 participants per cohort) were planned, including 9 HV cohorts, with dosing starting at 0.38 mg/kg and escalating through doses of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 mg/kg. For each participant (both Part A and Part B), clinical facility confinement was approximately 2 days, starting on Day 1 with dosing on Day 1 and discharge on Day 2. Two sentinel HV participants were dosed at 0.38 mg/kg (one ARC-AAT, one PBO) in part A; when no significant safety concerns were raised following Day 3 evaluation in these participants, the remaining four participants in Cohort 1 were treated. Part A participant data were collected at a single Phase 1 facility (Nucleus Networks, Melbourne Australia).

Part B of the study was initiated at the 2 mg/kg dose level with planned PiZZ patient cohorts at 2.0, 4.0, 6.0 and 8.0 mg/kg. Escalation to the next dose level proceeded based on all available safety data through Day 8 of the current cohort. Provision was made to halt dose

escalation if a dose-limiting toxicity (DLT) occurred, defined as a treatment-related adverse event (AE; rated as possibly or probably related) that may have posed an unacceptable risk to other participants or if target KD parameters were achieved. Part B participant data were collected during patient visits to participating clinical trial sites. Dose escalation through all HV cohorts was completed. In PiZZ individuals, the 2.0 mg/kg cohort was completed and 5 of 6 PiZZ individuals were enrolled into the 4 mg/kg cohort prior to termination of the study.

Study assessments

The safety analysis included all patients who received ARC-AAT or placebo. Safety measures included: (1) AEs; (2) physical examination; (3) vital signs; (4) 12-lead ECG measurement; (5) ECG telemetry (8 hours pre-dose to 24 hours post-dose); (6) pulmonary function testing (spirometry to include VC, FEV_1, FEV_1/VC) and DLCO, both conducted in accordance with ATS-ERS guidelines; (7) clinical laboratory tests (hematology, biochemistry, coagulation, urinalysis); (8) use of concomitant medications, and (9) recording reasons for treatment discontinuation. Scheduled evalutaions were performed by a blinded member of the site staff. Abnormalities in laboratory findings or other assessments that were deemed clinically significant by the study investigator and were initially detected during the study or were present at baseline and significantly worsened during the study were reported as AEs, whether or not they were considered drug-related by the study investigator. All AEs and SAEs were followed until resolution, until the condition stabilized, until the event was otherwise explained, or until the subject was lost to follow-up. Quantitative serum AAT was assessed by turbidometric and/or nephelometric methods (Roche Cobas® c501, ARCHITECT® ci16200™, BN™ II System). Serum AAT reduction is based on each individual's change from their baseline value with baseline calculated as the geometric mean

of three serum AAT measurements prior to receiving test article. Further details on when each assay was used and a comparison between platforms can be found in Figs. S3-S5. PK samples were analyzed using a validated hybridization-ligation method for the API and a validated LC/MS/MS method for the delivery excipient.

Investigational medicinal product

ARC-AAT Injection comprised API-AAT, the Active Pharmaceutical Ingredient consisting of a single cholesterol-conjugated unlocked nucleobase analog (chol-UNA) RNAi trigger targeting AAT mRNA supplied in a liquid solution, plus the delivery excipient ARC-EX1 containing hepatocyte-targeted N-acetylgalactosamine-conjugated melittin-like peptide supplied as a powder. The vials were prepared by an un-blinded pharmacist in the pharmacy under sterile conditions to yield a 2:1 ratio by weight (API-AAT:ARC-EX1) and then administered by a blinded site staff member as a single intravenous infusion at a rate of 20 mg/min. All subjects were treated with an oral antihistamine approximately 2 hours prior to infusion.

Objectives

Primary objectives were to determine the safety and tolerability of escalating doses of ARC-AAT, to evaluate PK of ARC-AAT components at different doses, and to determine the effect of ARC-AAT on circulating levels of AAT. Key secondary objectives included determining the dose levels achieving >30% KD and ≥ 90% KD of AAT on or before Day 22 (± 1 day) when compared to the pre-dose geometric mean baseline AAT level in HVs, determining the dose level achieving > 90% KD at Day 29 (± 1 day) in 50% of PiZZ

individuals and the time for serum AAT levels to return to baseline (± 15% from geometric mean baseline or > 90 mg/dL for HV).

Data handling and statistical analysis

No statistical methods were used to determine cohort sizes or total subjects planned for enrollment. Safety results were summarized by cohort and treatment group. The frequency of treatment-emergent AEs, SAEs, related AEs, related SAEs, and AEs leading to withdrawal, dose modification, or treatment discontinuation were summarized by dose and treatment group according to the latest version of MedDRA by System Organ Class (SOC) and Preferred Term. Plasma concentrations of ARC-AAT Injection constituents (MLP, chol-UNA) following a single dose of ARC-AAT injection at different dose levels were used to calculate the following ARC-AAT PK parameters: Cmax, tmax, AUC0-24, AUCinf, and t1/2. Pharmacokinetic parameters were determined using non-compartmental method(s) with Phoenix™ WinNonlin® Version 6.3 software (Copyright ©1998-2012, Certara L.P., distributed by Pharsight Corporation). Descriptive statistics of PK parameters included mean, standard deviation, and coefficient of variation. Pharmacodynamic (PD) analyses were performed using Microsoft Excel 2013 and GraphPad Prism software version 6.0. Descriptive statistics included geometric mean, mean, standard error of mean, and percent AAT serum concentration change from baseline.

Results

Healthy volunteer and PiZZ individual characteristics

Fifty-four HVs were enrolled with 36 receiving ARC-AAT and 18 receiving placebo. All HVs resided in Australia; characteristics are shown in Table 1. Eleven PiZZ AATD individuals were enrolled from patients screened in the United Kingdom, Australia, the Netherlands and Germany; their characteristics are shown in Table 1. PiZZ patients included in the study had minimal or no liver or lung disease. Baseline FEV1 and FibroScan® parameters for individual PiZZ patients are presented in Table 2. The mean PiZZ patient FEV1 at baseline was 85% (Range 72-93%). The mean baseline FibroScan® was 5.58 kPa (Range 4.0-6.6) which is consistent with very minimal or no liver fibrosis. Seven PiZZ individuals received ARC-AAT and 4 received placebo. Fig. S2 presents the Consort diagram for the study.

Safety, pharmacokinetics and pharmacodynamics

In both Part A (HV) and Part B (PiZZ), there were no deaths and no serious adverse events (SAEs) in subjects receiving ARC-AAT. There was one SAE of rhabdomyolysis in a subject receiving placebo. There were no AEs rated as severe in intensity in subjects receiving ARC-AAT or AEs leading to premature study discontinuation. The only AE showing a pattern indicating possible dose relatedness was chills, reported in 3 of 4 HVs at the 8 mg/kg dose and likely related to the infusion of ARC-AAT (Tables S1A, B). Headaches, muscle spasms, upper respiratory tract infection, nausea and dysmenorrhea were seen more frequently in healthy volunteer subjects receiving ARC-AAT. However, there is no clear mechanistic association between ARC-AAT and these symptoms. In the AATD patients, no AE occurred more than once in the seven subjects receiving ARC-AAT. A single AE with the preferred term of “asthma” was reported in an AATD patient receiving 2 mg/kg of ARC-AAT. This AE was reported as mild and in a patient with a previous history of asthma treated with

inhaled steroids and inhaled β2-receptor agonists. Symptoms began on Day 7 and were reported to last approximately 5 minutes, then resolving with use of inhaled beta-agonists. The study investigator reported this AE as not related to ARC-AAT but rather related to cold air exposure. This patient did not experience any meaningful adverse changes in FEV1 during the study. There were no clinically significant adverse changes in ECG, DLCO, or FEV_1 and no increased rate of pulmonary AEs in subjects receiving ARC-AAT. Baseline and post-dose values for FEV1 and select clinical laboratory assessments in HV subjects and PiZZ patients are presented in Table S2A, B. The study was terminated early due to toxicity findings related to the delivery vehicle (ARC-EX1) seen in a non-human primate study.

All subjects randomized to ARC-AAT in Part A had PK concentrations reported for all samples collected. One HV dosed at 8.0 mg/kg did not receive a full dose (<50%) and thus was excluded from the PK summary. The PK parameters in HVs and patients are shown in the Table S3; overall results indicated that the API circulates with a short half-life of approximately 4 hours. The delivery excipient, ARC-EX1, demonstrated a half-life of 8-10 hours. Exposure increased linearly with dose.

There was a clear PD dose response to ARC-AAT when considering serum AAT levels, with prominent (>30%) reductions seen at all doses ≥ 2mg/kg (Fig. 1; Fig. S6). The greatest reductions were seen at approximately four weeks post-dose. The time to reach maximum serum AAT reduction may be in part due to the 5-7 day half-life of serum AAT. Reductions in serum AAT levels achieved in Cohort 3 (2.0 mg/kg) were sufficient to transition to Part B of the study based on the criteria pre-defined in the protocol. Continuing dose escalation in HVs showed a dose-related response for reduction in serum AAT levels, with mean relative maximum reductions of 88.3% seen at the 8.0 mg/kg dose level (Fig. 1; Table 3; Fig S6). The relative percentage reduction in AAT for PiZZ patients receiving 2.0 mg/kg and 4.0 mg/kg ARC-AAT was similar to that in the healthy subjects at the same dose

level (Fig. 1). The maximum reduction in a single PiZZ patient was 78.8% at 4.0 mg/kg ARC-AAT, compared with 76.1% in HVs at this dose. Although the percentage change in serum AAT changes were similar in HV and PiZZ subjects, the baseline and maximum post-dose reduction absolute values were different. The average baseline serum AAT concentration for HV was 144.3 mg/dL (range: 101-216) and for PiZZ was 25.3 mg/dL (range: 20.3-35.0) (Fig. S7). The lowest serum AAT concentration reached was 13.2 and 5.9 mg/dL in HV and PiZZ subjects, respectively.

Dose range to achieve target knockdown and duration of knockdown

Greater than 30% KD was achieved in HVs at doses of 3.0 mg/kg and above, while 89.8% was the maximum KD achieved at 8.0 mg/kg in any subject, with a clear dose response and plateau effect at doses ≥6.0 mg/kg (Fig. 1; Table 3). Similar effects in PiZZ patients were observed (Fig. 1), although the 90% KD target was not achieved, presumably due to early termination of dose escalation. The duration of ≥ 30% KD was around 8 weeks in HVs at optimal (≥6 mg/kg) doses, and KD durations were similar in PiZZ and HVs at equivalent doses (Fig. 1).

Discussion

The main finding from this randomized, controlled phase 1 study of a hepatocyte-targeted RNAi therapeutic was a clear dose response in serum AAT knockdown, with a mean maximum knockdown of 88.3% observed at the highest (8 mg/kg) dose level in healthy volunteers. A dose response was also seen in PiZZ individuals with maximum serum AAT knockdown of 78.8% at the 4 mg/kg dose level. The level of knockdown seen in healthy

volunteers and in PiZZ individuals was similar at equivalent doses. In this study, ARC-AAT was safe, with no SAEs observed in actively treated subjects while other AEs observed were relatively unremarkable (Tables S1A, B).

Circulating serum AAT levels in healthy adults will generally exceed 90 mg/dL and represent a combination of AAT secreted from the liver and extrahepatic sources [16]. Based on the plateau in maximum AAT reduction (Fig. 1), it appears that approximately 90% of circulating AAT is produced in the liver in phenotypically normal adults. This reduction is similar to that observed in transgenic mice transfected with the human PiZ AAT gene when they are treated with maximal doses of a hepatic-directed siRNA [17]. Due to the premature discontinuation of this clinical trial because of an unexpected animal toxicity seen with a different compound sharing the same ARC-EX1 delivery excipient, we were unable to evaluate the full dose range in PiZZ AATD patients as planned. As expected from the experience in PiZ transgenic mice and HVs, ARC-AAT reduced circulating AAT serum concentrations in AATD patients. Comparing percentage reductions for AATD individuals and HVs (Fig. 1; Table 3), PiZZ individuals responded at least as well to ARC-AAT with respect to both depth and duration of AAT knockdown. Similar results have been reported by other groups developing siRNA therapeutics targeting hepatic alpha-1 antitrypsin production [18].

The method used for AAT knockdown involved the active agent (API) and a delivery excipient (ARC-EX1). The API consisted of a single cholesterol-conjugated unlocked nucleobase analog (chol-UNA) targeting AAT mRNA. The RNAi trigger was conjugated to cholesterol to increase the uptake of API in the liver by endocytosis while the delivery excipient, also targeted to the liver by conjugation to N-acetylgalactosamine, enabled endosomal escape of the siRNA into the cytoplasm where RNAi occurs [19]. This approach was effective in that it resulted in significant knockdown of AAT in both HVs and PiZZ

patients. This indicates that RNAi is a viable strategy for further development for the reduction of AAT hepatic synthesis.

AATD predisposes to emphysema because of an inadequate protease screen in the lung, leaving neutrophil elastase unchecked and resulting in loss of alveolar walls. In contrast to the toxic loss of function that defines the emphysema risk in AATD, the pathogenesis of liver disease relates to a toxic gain of function related to the inability to secrete polymerized AAT that accumulates in the hepatocyte with specific alpha-1 antitrypsin variants, leading to endoplasmic reticulum stress, fibrosis, and ultimately cirrhosis. A recent cross-sectional study of adult AATD patients demonstrated that advanced fibrosis can be present without clinical manifestations of disease [5]. However, liver fibrosis and even cirrhosis may regress with removal of the hepatic insult which supports silencing hepatic alpha-1 antitrypsin production in patients with liver disease [20]. Survival in AATD patients is increasing, likely due to less smoking, reduced exposure to inhaled environmental toxins and better pulmonary care [21]. With improvement in lung disease-related mortality, it seems likely that liver disease will be increasingly recognized in PiZZ adults in the future. The only options currently available for patients with AATD-associated liver disease include standard supportive care (e.g. avoidance of hepatic toxins and vaccination) and liver transplant. The development of RNAi therapeutics targeting the hepatic production of Z-AAT protein would represent a novel approach to preventing and potentially reversing AATD-associated liver disease. The results from this first-in-human study are encouraging in that they demonstrate potent and prolonged reduction of hepatic AAT synthesis as measured by serum AAT levels. This first-in-human study of an RNAi therapeutic may represent a first step in the development of a new class of hepatocyte-targeted therapeutics for the treatment of AATD-associated liver disease.

Detecting AATD liver disease is generally based on clinical suspicion. Optimal strategies to image the liver for early detection of fibrosis in AATD, as are recognized in

many other fibrotic liver conditions [22], are not yet established [23]. Subjects included in our study did not have clinically significant liver disease, based on either clinical evaluation or on transient elastography (FibroScan®) at screening. This and the phase 1 single escalating dose design precludes any conclusions being drawn about the efficacy of ARC-AAT in reducing liver pathology. Future multi-dose studies of siRNA drug candidates for AATD liver disease will likely need to demonstrate a histologic benefit based on liver biopsy, a reduction in the rates of liver-related events (e.g. variceal bleed or transplant) or a major effect on surrogate outcomes such as elastography or other biomarkers. A small study of the enhanced liver fibrosis panel (ELF) as a biomarker in AATD patients has been published [24], but further work is needed to understand if this would be a viable, less invasive alternative to biopsy.

With termination of all ARC-EX1 containing programs by the study sponsor due to ARC-EX1 delivery excipient-related toxicity findings in a non-clinical study, further development of RNAi mediated knockdown of AAT is ongoing utilizing strategies which do not require a delivery excipient. In addition to an expected reduction in toxicity, this newer method in pre-clinical development can potentially use subcutaneous rather than intravenous administration.

One concern facing this strategy is the potential that knockdown of AAT may increase the risk of lung disease in AATD patients if used chronically, since participants with null genotypes who produce no AAT in any tissue exhibit more severe emphysema [25]. In this study, no significant adverse changes in lung function were seen, although we recognize that the short duration of treatment was insufficient to fully examine for changes in lung function. To mitigate concerns of RNAi-induced reductions in serum AAT in AATD participants with pre-existing emphysema, AAT augmentation therapy could be used concomitantly with liver-targeted siRNA therapeutics intended to treat liver disease [26]. It

should also be emphasized that the AATD patients treated in this study by design were relatively healthy with minimal lung or liver disease based on standard non-invasive measures. The safety and pharmacodynamic response of this approach will need to be evaluated in patients with more advanced lung and liver disease.

Strengths of our study include the large number of HVs tested, the wide range of doses studied and the inclusion of PiZZ subjects in the age group where such a product would likely be used. Limitations include the early study termination, short duration of treatment and the lack of liver biopsy outcomes in participants. However, we believe that liver biopsy would not have been appropriate prior to phase 2 work due to its invasive nature, its corresponding procedural risk, and uncertain therapeutic benefit in this single dose phase 1 study that enrolled relatively healthy PiZZ subjects.

In conclusion, RNAi can be used to reduce hepatic production of AAT based on dose responsive reductions in serum AAT levels seen after administration of single doses of ARC-AAT, and siRNA shows promise for treatment of AATD-associated liver disease.

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Figure Legends

Title:

Fig. 1: Serum AAT dose response to ARC-AAT in healthy volunteers and PiZZ subjects.

Legend:

A single intravenous dose of (A) 0.38 to 8.0 mg/kg ARC-AAT (Cohorts 1-3g) was given to healthy volunteers, and (B) 2 and 4 mg/kg ARC-AAT (cohorts 4 and 5) given to PiZZ subjects. Serum AAT was measured weekly for the first four-weeks and then bi-weekly until serum concentrations returned to baseline. (C) Duration of knockdown in healthy volunteers and PiZZ subjects was similar. Mean line plotted when n>1 at time point. Subjects receiving ARC-AAT per dose level, n=3-4 (healthy volunteers), n=3 (PiZZ), receiving placebo, n=18 (healthy volunteers), n=4 (PiZZ). Legend doses are in mg/kg. Data represents the mean ± SEM. Abbreviations: PBO, Placebo; HV, healthy volunteers; ZZ, PiZZ subjects.

Table 1: Characteristics of the participants

Legend: Numerical values are shown as mean (SD) and frequency variables as n (%)

Dose (mg/kg) N = HV, PiZZ		**0.38 n=4**	**1.0 n=4**	**2.0 n=4, 4**	**3.0 n=4**	**4.0 n=4, 3**	**5.0 n=4**	**6.0 n=4**	**7.0 n=4**	**8.0 n=4**	**Total n=36, 7**	**Placebo n=18, 4**	**All n=54, 11**
HVs	Mean age in years (SD)	39.0 (9.8)	25.0 (4.5)	30.0 (7.8)	29.5 (10.7)	32.3 (5.6)	23.8 (4.1)	32.5 (15.3)	26.0 (5.0)	25.0 (1.8)	29.2 (8.6)	25.4 (4.8)	28.0 (7.7)
	Mean BMI (SD)	25.28 (2.92)	21.48 (1.36)	23.10 (3.38)	23.75 (2.70)	23.45 (2.01)	21.75 (2.08)	23.85 (1.61)	22.53 (2.03)	23.65 (3.47)	23.20 (2.46)	23.38 (2.37)	23.26 (2.41)
	Male	3 (75%)	0	2 (50%)	3 (75%)	3 (75%)	0	1 (25%)	1 (25%)	2 (50%)	15 (42%)	6 (33%)	21 (39%)
	Mean baseline serum AAT, mg/dL (SD)	119 (12.2)	154 (29.1)	119 (15.6)	137 (12.1)	140 (20.5)	183 (36.4)	151 (27.1)	164 (22.0)	141 (17.3)	145 (29.3)	143 (23.0)	145 (27.3)
PiZZ patients	Mean age in years (SD)			59.0 (1.8)		64.3 (3.8)					61.3 (3.8)	58.8 (9.0)	60.4 (5.9)
	Mean BMI (SD)			27.50 (3.56)		24.10 (2.63)					26.04 (3.46)	26.43 (2.73)	26.18 (3.07)
	Male			1(25%)		2 (67%)						1 (25%)	4 (36%)
	Mean Baseline FEV1%* (Range)			85 (72-93)		91 (78-104)					87.57 (72-104)	93.25 (80-104)	89.64 (72-104)
	Mean baseline DLCO%** (Range)			65.75 (57-73)		63.33 (51-73)					64.71 (51-73)	68.5 (43-105)	66.09 (43-105)
	Mean baseline serum AAT, mg/dL (SD)			25.2 (6.1)		27.4 (2.9)					26.2 (5.0)	24.3 (4.4)	25.5 (4.8)
	Mean baseline FibroScan® kPa (Range)			5.58 (4.0-6.6)		5.57 (5.0-6.1)					5.57 (4.0-6.6)	5.58 (4.1-7.5)	5.57 (4.0-7.5)

Table 2: Individual PiZZ patients baseline measures of lung and liver disease severity.

Legend: FEV1% = forced expiratory volume in one second as a % of predicted

PiZZ Patient	Gender	Age	Baseline FEV1%	Baseline FibroScan® (kPa)	ALT (U/L) Day 1 pre-dose
Patient 1	Female	58	93	5.5	19
Patient 2	Female	64	80	5.5	17
Patient 3	Female	57	72	6.6	17
Patient 4	Male	61	84	6.2	19
Patient 5	Female	60	91	4.0	27
Patient 6	Female	59	97	5.2	23
Patient 7	Male	66	78	5.6	17
Patient 8	Female	66	92	4.1	12
Patient 9	Male	60	104	5.0	38
Patient 10	Female	67	91	6.1	17
Patient 11	Male	46	104	7.5	24

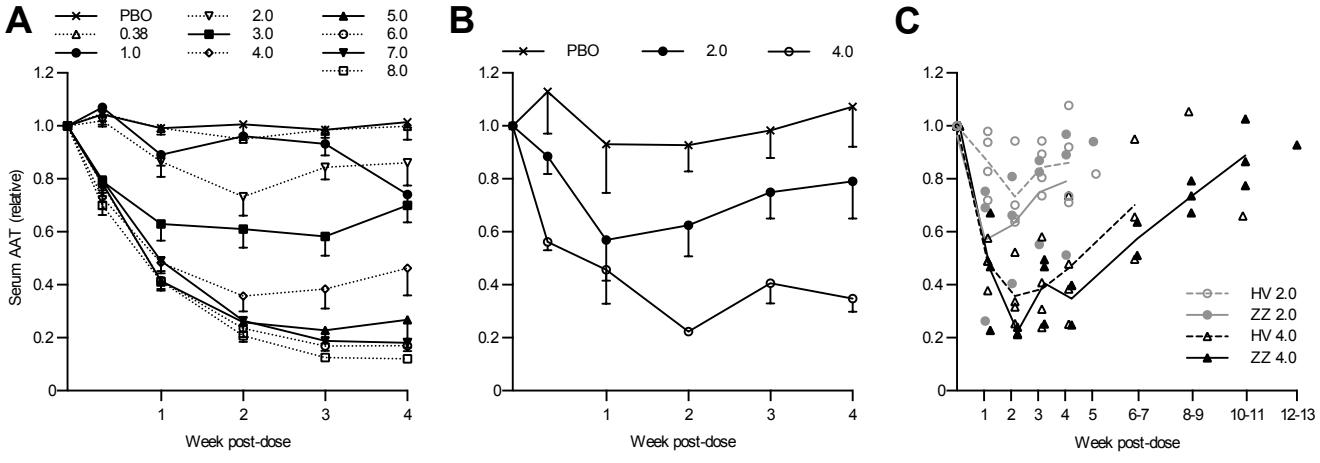
Table 3: Maximum serum AAT KD across dose levels in healthy volunteers and PiZZ individuals

Legend: Abbreviations: KD, knockdown; HV, healthy volunteers; ZZ, PiZZ patients

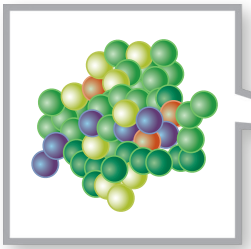
Dose Level (mg/kg)	**PBO**	**0.38**	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**	**8.0**
HV (n)	18	4	4	4	4	4	4	4	3	3
Max KD (%)	24.8	9.30	31.9	36.3	61.0	76.1	86.7	87.1	85.1	89.8
Mean Max KD (%) (SEM)	8.44 (1.80)	6.55 (1.49)	25.9 (3.29)	26.7 (7.14)	45.3 (6.80)	64.8 (6.07)	78.1 (4.38)	83.3 (1.93)	82.6 (1.31)	88.3 (0.769)
***P* value**	N/A	0.6363	0.0004	0.0014	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
ZZ	4	-	-	3	-	3	-	-	-	-
Max KD (%)	46.6	-	-	73.7	-	78.8	-	-	-	-
Mean Max KD (%) (SEM)	21.9 (9.81)	-	-	44.1 (15.1)	-	78.1 (0.481)	-	-	-	-
***P* value**	N/A	-	-	0.2524	-	0.0047	-	-	-	-

Article Highlights:

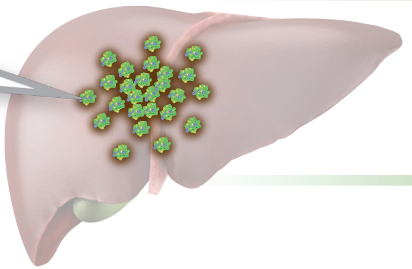
- Alpha-1 antitrypsin deficiency is associated with liver fibrosis and cirrhosis
- RNAi drugs might benefit patients with alpha-1 antitrypsin deficiency
- ARC-AAT reduced alpha-1 antitrypsin protein in study participants


A
PBO
0.38
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Serum AAT (relative)
Week post-dose
B
PBO
2.0
4.0
Week post-dose
C
HV 2.0
ZZ 2.0
HV 4.0
ZZ 4.0
Week post-dose

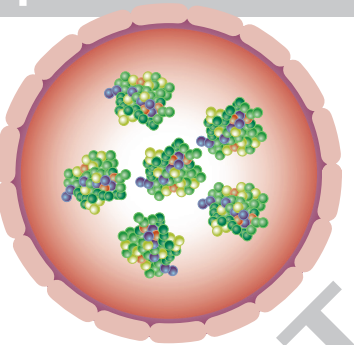
Alpha-1 Antitrypsin protein



Normal liver

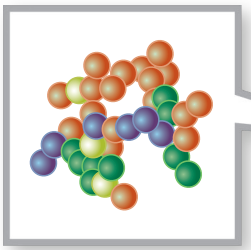


Normal secretion into the blood

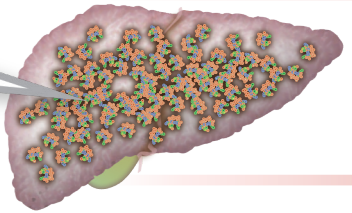


Normal serum levels of normal protein

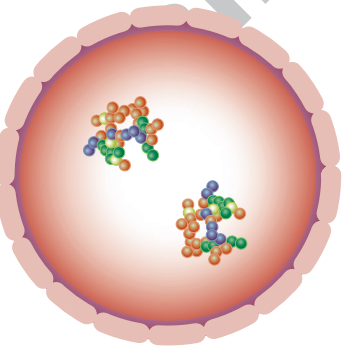
Misfolded Alpha-1 Antitrypsin protein



Liver affected by AATD



Abnormal secretion into the blood



Low serum levels of abnormal protein

High accumulation of misfolded Alpha-1 Antitrypsin protein leads to liver injury

Liver-targeted siRNA

Inhibits liver AAT protein production in healthy volunteers and AATD patients