PROTOCOL NUMBER:	**AROAAT2001**
STUDY TITLE:	**A Placebo-Controlled, Multi-dose, Phase 2 Study to Determine the Safety, Tolerability and Pharmacodynamic Effect of Fazirsiran (TAK-999, ARO-AAT) in Patients with Alpha-1 Antitrypsin Deficiency (AATD) [SEQUOIA]**
DRUG (Active):	**Fazirsiran (TAK-999, also referred to as ARO-AAT) Injection**
ROUTE:	**Subcutaneous Injection**
STUDY DESIGN:	**A multi-center, multi-dose placebo-controlled Phase 2 study will be conducted to evaluate the safety, tolerability, and pharmacodynamic effect of the investigational product, fazirsiran (TAK-999, ARO-AAT), administered subcutaneously to patients with alpha-1 antitrypsin deficiency**
SPONSOR:	**Arrowhead Pharmaceuticals, Inc.** **177 East Colorado Blvd., Suite 700** **Pasadena, CA 91105** **Telephone: +1 626 304-3400** **Facsimile: +1 626 304-3401**
EudraCT Number:	**2018-003385-14**
Version:	**6.0 Global, 29 August 2022**

1. PROTOCOL SYNOPSIS

Study Title: A Placebo-Controlled, Multi-dose Phase 2 Study to Determine the Safety, Tolerability and Pharmacodynamic Effect of Fazirsiran (TAK-999, ARO-AAT) in Patients with Alpha-1 Antitrypsin Deficiency (AATD) [SEQUOIA]

Study Number: AROAAT2001

Phase: Phase 2

Location: Multiple sites in the United States and Europe.

Study Treatments:

There will be 2 study treatments; one active and one placebo.

Active: Fazirsiran Injection (also referred to as ARO-AAT Injection or TAK-999 Injection)

The active pharmaceutical ingredient (API) fazirsiran is a synthetic, double-stranded, small interfering RNA oligonucleotide (siRNA) duplex conjugated to an N-acetyl-galactosamine targeting ligand to facilitate hepatocyte delivery.

Placebo (PBO)

The placebo used in this study is normal saline (0.9%) administered subcutaneously, volume matched to the corresponding fazirsiran dose volume.

Primary Objective:

- To select a single dose for use in later stage development based on a combined evaluation of safety and pharmacodynamic (PD) effects of fazirsiran

Primary Endpoint:

- Percent change from baseline at Week 16 in serum Z-AAT

Secondary Endpoints:

- Subject incidence of treatment-emergent adverse events (AEs)
- Absolute and percent change from baseline in total liver Z-AAT (insoluble + soluble) protein at post-dose biopsy visit
- Absolute and percent change from baseline in liver Z-AAT soluble protein at post-dose biopsy visit

- Absolute and percent change from baseline in liver Z-AAT insoluble protein at post-dose biopsy visit
- Absolute and percent change from baseline in liver function tests including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, gamma glutamyl transferase (GGT), total bilirubin, direct bilirubin, and international normalized ratio (INR) at Week 16 and over time through End of Study (EOS)
- Absolute and percent change in serum Z-AAT over time through EOS
- Change over time in pharmacokinetic (PK) measurements of fazirsiran at timepoints specified in the Schedule of Assessments (SOA)
- Incidence of anti-drug antibodies (ADAs) to fazirsiran
- Change from baseline in Metavir fibrosis stage at post-dose biopsy

Exploratory Endpoints:

- Change from baseline in liver histology (e.g., globules, inflammation, steatosis) at post-dose biopsy
- Change from baseline in hepatic *SERPINA1* messenger RNA (mRNA) expression at post-dose biopsy
- Change from baseline in liver fibrosis gene expression at post-biopsy
- Absolute and percent change from baseline in serum PRO-C3 at Week 16 and over time through EOS
- Change from baseline in noninvasive scoring systems for fibrosis, including aspartate aminotransferase-to-platelet ratio index (APRI) and Fibrosis-4 index (FIB-4), at Week 16 and over time through EOS
- Changes from baseline in FibroScan® over time through EOS
- Change from baseline in hepatic stiffness based on Magnetic Resonance Elastography (MRE) (if available) over time through EOS
- Change from baseline in hepatic fat content based on magnetic resonance imaging (MRI) (MRI-proton density fat fraction [MRI-PDFF] if available) over time and through EOS
- Change from baseline in hepatic iron content based on MRI (if available) over time and through EOS

- Change from baseline in markers of liver fibrosis, globules, and iron content using biomarkers (e.g., PRO-C6) and special stains and imaging (Masson's Trichrome, Sirius Red, Iron, PAS-D), which may include histologic morphometric analysis (if scientifically feasible and sufficient sample available), over time and through EOS

Study Population/Patient Number: This study will be conducted in patients homozygous for the Z mutation (PiZZ) with confirmed AATD. Both males and females are eligible, ages 18 to 75 years. In total, the study will consist of approximately 36 patients. A minimum of 24 out of 36 patients must have evidence of fibrosis on the liver biopsy at Screening.

Study Key Inclusion/Exclusion Criteria: Patients must meet the following key eligibility criteria (per Sections 7.2 and 7.3) for study entry: no evidence of definitive cirrhosis; non-smoking status (defined as does not smoke cigarettes daily for at least 12 months); platelet count $\geq 150 \times 10^9$/L; forced expiratory volume in one second (FEV_1) $\geq 65\%$.

Study Design/Methods: A multi-center, multi-dose placebo-controlled Phase 2 study will be conducted to evaluate the safety, efficacy, and tolerability of the investigational product, fazirsiran (TAK-999, ARO-AAT), administered subcutaneously to patients with AATD.

Study Details

The study will test 3 fazirsiran dose levels compared to placebo. Patients who have signed an Institutional Review Board (IRB)/Ethics Committee (EC)-approved informed consent and have met all the protocol eligibility criteria during Screening will be assigned to one of 3 cohorts and randomized 2:1 (active: placebo) within each cohort. The 3 cohorts of the study are as follows:

- **Cohort 1:** 25 mg dose of fazirsiran or placebo
- **Cohort 2:** 100 mg dose of fazirsiran or placebo
- **Cohort 3:** 200 mg dose of fazirsiran or placebo

Within each cohort, requirements for biopsies, dosing schedules, and SOA will be determined based on the patient's fibrosis score during Screening.

Patients with no evidence of fibrosis

Patients who have a documented biopsy showing no evidence of fibrosis within 1 year of the Screening visit will not require liver biopsy at any point during the study.

Patients without fibrosis at Screening will receive 2 doses of fazirsiran or placebo on Day 1 and Week 4, as per the SOA. Following their Week 4 dose, these patients remain in the study with regular visits per the SOA until Week 64.

Patients with evidence of fibrosis

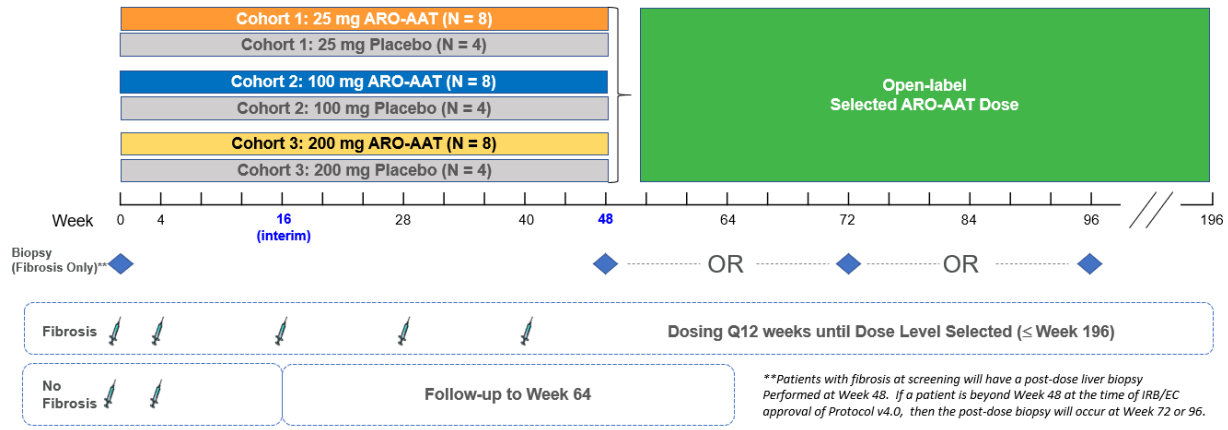
Patients who have a pre-dose biopsy showing evidence of fibrosis (without definitive cirrhosis) during Screening will have a post-dose biopsy performed at Week 48. If a patient is beyond Week 48 at the time of IRB/EC approval of Protocol v4.0, then the post-dose biopsy will occur at Week 72 or 96. The study will end after the last patient with fibrosis receives their 10th dose (Week 100 visit) or until a long-term extension study is available at the subject's study site, whichever comes first.

Patients with evidence of fibrosis at Screening will receive a dose on Day 1, Week 4, and Week 16, then every 12 weeks for up to 18 doses total.

Dose Evaluation and Selection

After all enrolled patients completed the Week 16 visit, an Interim Analysis was performed to select a single dose level (25, 100, or 200 mg) for the open-label phase of the study. Based on cumulative safety, efficacy, and pharmacodynamic (PD) data from the fazirsiran clinical program (clinical studies AROAAT1001, AROAAT2001, and AROAAT2002), a fazirsiran dose (200 mg) was selected by the Sponsor. All patients with fibrosis at Screening who completed the post-dose biopsy visit will receive fazirsiran at the selected dose for the duration of the study (open-label phase) but will remain blinded to the randomized treatment assignment.

Study Schema



Number of Doses: The number of fazirsiran doses administered to each patient varies, but a minimum of 2 and a maximum of 18 total doses will be administered to each patient in the study. **Patients without fibrosis** will receive 2 doses (one at Day 1 and one at Week 4). **Patients with fibrosis** will receive up to 18 doses (Day 1, Week 4, Week 16 followed by a dose once every 12 weeks). All patients will discontinue dosing once the last enrolled patient with fibrosis receives their 10th dose (Week 100 visit) or until a long-term extension study is available at the subject's study site, whichever comes first.

Study Duration: For patients without fibrosis, the duration of the study is approximately 72 weeks, with an 8-week Screening period and a 64-week study follow-up period. For patients with fibrosis, the duration of the study is up to 216 weeks, with an 8-week Screening period, a 196-week study dosing period (if all 18 doses are received), followed by a Study Completion visit performed within 4 to 12 weeks from the dosing visit.

Study Assessments:

Patients will undergo the following evaluations at regular intervals during the study (refer to the SOA): medical history, physical examinations, vital sign measurements (blood pressure [BP], temperature, heart rate [HR], respiratory rate [RR]), weight (at baseline), AE monitoring, electrocardiograms (ECGs), urine pregnancy test/follicle-stimulating hormone (FSH) (females), concurrent medication, pulmonary function testing (spirometry including vital capacity [VC], forced vital capacity (FVC), FEV_1, FEV_1/VC, FEV_1/FVC, and diffusing capacity for carbon monoxide [DLCO]) and sample collection for hematology, coagulation, biochemistry, lipids, cardiac troponin, urinalysis, urine cotinine, ADAs, drug screens, serum alpha-1 antitrypsin levels, serum fibrosis biomarkers, liver biopsy, MRI (where feasible and available), and FibroScan® (where available).

Study visits will occur during the Screening window (Day -60 to -1), and as per the SOA. A telephone follow-up will occur 90 days after the last dose (± 5) to verify compliance with contraceptive measures and absence of any known pregnancy. Clinically significant changes including AE will be followed until resolution is achieved, or until medically stable or the event is otherwise explained, or until the patient is lost to follow-up.

Unless otherwise noted, baseline values will be those assessments obtained pre-dose within the closest proximity to the first dose.

Safety Assessments will be performed at specified timepoints per the SOA and will include the following:

- Vital signs: Resting HR, seated or semi-supine (same position each time is preferred) systolic/diastolic BP, RR, and temperature
- Clinical laboratory measurements (e.g., biochemistry, hematology, cardiac troponin, lipids, coagulation, FSH, urine pregnancy test [women of childbearing potential {WOCBP}], urine cotinine, and urinalysis)
- Resting ECG measurements (measured after patient is supine or semi-supine [same position each time is preferred] for at least 3 minutes)
- At each visit, patients will be asked about concomitant medications/therapy and will be instructed to volunteer any information regarding AEs and serious adverse events (SAEs) that they may have experienced. Any known untoward event that occurs beyond the AE reporting period that the Investigator(s) considers an SAE and possibly related to study treatment will be reported to the Sponsor

- 90-day, post-last dose follow-up phone call to assess for pregnancy occurrence
- Physical examination (symptom directed as described in the SOA)
- Pulmonary Function Testing including spirometry (including VC, FVC, FEV_1, FEV_1/VC, and FEV_1/FVC) and DLCO
- Use of augmentation treatment at any time throughout the study
- Time from baseline to initiation of augmentation therapy
- Number and severity of chronic obstructive pulmonary disease (COPD) exacerbations based on Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria in active versus placebo will be evaluated as an AE of special interest

The AE reporting period for an enrolled patient will begin when the patient provides informed consent. Treatment-emergent AEs will be those defined as following dose administration, or in the event onset preceded dose administration, those AEs with severity or frequency increasing post-dose. All AEs that occur during the AE reporting period specified in the protocol must be reported, regardless of the relationship of the AE to study treatment. For this trial, the Investigator should evaluate the relatedness of an AE to investigational product using 3 categories: Not Related, Possibly Related, and Probably Related. Laboratory abnormalities will be reported as an AE if considered clinically significant by the Investigator or if there are clinical sequelae. Any known SAE that occurs beyond the AE reporting period that the Investigator considers possibly or probably related to study treatment will be reported.

An independent Data Safety Monitoring Board (DSMB) will be established to review safety data at regular intervals. The safety of fazirsiran will be reviewed by the DSMB after at least 18 enrolled patients have received at least one dose of study drug and completed the Week 16 visit. An independent statistical center (ISC) will provide unblinded comparative safety summaries to the DSMB in a restricted manner. The Sponsor will not have access to the unblinded summaries. In addition to the routine safety summaries, the DSMB safety review will include evaluation of imbalances in clinically significant adverse changes in FEV_1, DLCO, or pulmonary related AEs. The DSMB may be asked to review safety data at additional unscheduled meetings. The DSMB may also make recommendations regarding cessation of dosing in individual patients, modification of individual study cohorts, conduct of the study, and/or study design. The specific responsibilities and composition of the DSMB will be outlined in the DSMB Charter.

Pharmacodynamic and Efficacy Assessments:

The following PD measures and diagnostic studies will be collected for each dose and treatment group as per the SOA:

- Quantitative and/or % change in serum alpha-1 antitrypsin levels (using Z-specific and/or clinical assay)
- Percent and absolute change in liver total (soluble plus insoluble) mutant AAT protein from Z allele (Z-AAT) protein levels
- Percent and absolute change in liver Z-AAT soluble protein levels
- Percent and absolute change in liver Z-AAT insoluble protein levels
- Percent change in expression of liver fibrosis associated genes
- Percent change in *SERPINA1* messenger RNA (mRNA) levels
- Change in measures of fibrosis (Metavir fibrosis stage)
- Change in liver histology (e.g., globules, inflammation, steatosis)
- Measures of hepatic injury including ALT, GGT, and AST
- Change in MRI (where available and if feasible)
- Change in FibroScan® (where available)
- Serum fibrosis biomarkers (PRO-C3/PRO-C6), FIB-4, APRI

Pharmacokinetic Assessments:

Blood samples will be collected from all enrolled patients for sparse sampling plasma pharmacokinetic (PK) analysis after dose 1 (all patients) and 3 (fibrosis subset) per the SOA.

Immunogenicity Assessments:

Emergence of ADAs will be evaluated with samples drawn per the SOA.

Statistical Considerations: The objective of this Phase 2 study is to evaluate 3 dose levels relative to placebo and select a dose level to be used in the open-label phase of this study and in later stages of development based on the evaluation of safety, as well as percent change from baseline in serum Z-AAT levels. The primary efficacy analysis, executed after the last patient completes Week 16 visit, will evaluate the difference in mean percent change from baseline at Week 16 between each active dose group and the pooled placebo groups. The primary efficacy analysis, performed using the mixed model repeated measures (MMRM) approach, will be

executed in all randomized patients who receive at least one dose of study treatment (Full Analysis Set), regardless of the total number of received doses. With a total sample size of approximately 36, the study has 99% global power to declare at least one treatment dose group as different from placebo, assuming the treatment difference from placebo of at least 70 percent points (SD=35) and using a one-sided 2.5% level of significance with Hochberg's adjustment for multiplicity of testing. Details of planned statistical analysis will be provided in the statistical analysis plan (SAP).

Extended Efficacy Analysis will be performed after the last patient with fibrosis reaches the post-baseline biopsy visit. Patients will receive the treatment dose selected following the Week 16 Interim Analysis, only after the patient has completed the post-baseline biopsy visit (Week 48, 72, or 96). Final Analysis will be performed after all patients complete either an Early Termination or Study Completion visit and database lock.

Treatment Stopping and Study Modification Rules:

A decision to stop the trial early or discontinue drug in an individual patient or group of patients **may** be indicated based on any of the following:

1. In the case of 2 or more similar SAEs both considered at least possibly related to fazirsiran, the DSMB will meet within 3 days of the DSMB being notified of the second event and within the timeframe of required regulatory agency notification. The DSMB will review available aggregated data to determine if the study remains safe to proceed, should be discontinued, or should continue but with amendments.

2. Evaluation and fazirsiran study modification/discontinuation rules for elevated ALTs or worsening hepatic function (i.e., cirrhosis) and drug induced liver injury (DILI) are provided in Appendix 2.

3. Evaluation and fazirsiran study modification/discontinuation rules for declines in pulmonary function are provided in Appendix 3.

Table 1: Schedule of Assessments for Patients Without Fibrosis at Screening

Assessment	Screen		Day 1 (Dosing Day)	24 hrs After Day 1 Dose	Week 2	Week 4	24 hrs After Day 29 Dose	Week 6	Week 16	Follow-Up Period				Early Termination [5]
										Week 28	Week 40	Week 52	Week 64: Study Completion	
	Required Prior to Day 1				Day 15	Day 29 (Dosing Day)		Day 43	Day 113	Day 197	Day 281	Day 365	Day 449	
Windows (in days)	-60 to -1	-30 to -1		+1	± 5	± 5	+1	± 5	± 14	± 14	± 14	± 14	± 14	
Informed Consent	X													
Eligibility Criteria	X													
Demographics/Medical History	X													
PiZZ Genotype	X													
Height, Weight, & BMI	X													
Weight only			X			X			X	X	X	X	X	X
Urine Cotinine	X								X					X
Urine Drug Screen	X													
Hepatitis/HIV	X													
PFT (Spirometry and DLCO) [1]		X	Pre-dose X			Pre-dose X			X	X [1]	X	X [1]	X	X
FibroScan®	X												X	X
MRI (PDFF, Fe, MRE) [2]	X												X	X
Liver Biopsy [6]	X													
Vital Signs (BP, temperature, HR, RR)	X		Pre-dose & 1 hr post-dose X	X	X	Pre-dose & 1 hr post-dose X	X	X	X	X	X	X	X	X
Physical Exam [3]	X		Pre-dose X	X	X	Pre-dose X	X	X	X	X	X	X	X	X
ECG [7]	X		Pre-dose X			Pre-dose X			X	X [7]	X	X [7]	X	X
Urine Pregnancy Test [8]	X		Pre-dose X			Pre-dose X			X	X	X	X	X	X

Table 1: Schedule of Assessments for Patients Without Fibrosis at Screening (continued)

Assessment	Screen		Day 1 (Dosing Day)	24 hrs After Day 1 Dose	Week 2	Week 4	24 hrs After Day 29 Dose	Week 6	Week 16	Follow-Up Period				Early Termination [5]
										Week 28	Week 40	Week 52	Week 64: Study Completion	
	Required Prior to Day 1				Day 15	Day 29 (Dosing Day)		Day 43	Day 113	Day 197	Day 281	Day 365	Day 449	
Windows (in days)	-60 to -1	-30 to -1		+1	± 5	± 5	+ 1	± 14	± 14	± 14	± 14	± 14	± 14	
FSH (post-menopausal females)	X													
Clinical Labs (heme, coag, trop, chem, lipids, urinalysis) [4]	X		Pre-dose X	X	X	Pre-dose X	X	X	X	X	X	X	X	X
PRO-C3/PRO-C6	X								X					
Calculate APRI			X						X		X	X	X	X
Anti-drug Antibodies	X		Pre-dose X			Pre-dose X			X	X	X			
Z-AAT Level	X		Pre-dose X		X	Pre-dose X		X	X	X	X	X	X	X
Serum Alpha-1 Antitrypsin Level	X		Pre-dose X		X	Pre-dose X		X	X	X	X	X	X	X
Concomitant Meds/Therapies	X		X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X		X	X	X	X	X	X	X	X	X	X	X	X
Study Treatment (on Day 1 and Week 4)			X			X								
Pharmacokinetics (PK)			Pre-dose, 1 hr, & 2 hr post-dose X	X										

1. Pulmonary function testing includes: Spirometry including VC, FVC, FEV_1, FEV_1/VC, FEV_1/FVC, and DLCO. PFTs at Screening must be performed within the Day -30 to Day -1 window. Pertinent study FEV_1 will be based on post-bronchodilation value. Spirometry and DLCO may be repeated once per timepoint. Spirometry and DLCO should also be conducted any time a patient experiences a COPD exacerbation. **DLCO is optional at the Week 28 and Week 52 visit.**
2. MRI hepatoscan will include magnetic resonance elastography (MRE), hepatic fat content by MRI-PDFF (or equivalent), and hepatic iron content. MRI should always be completed before biopsy (if performed on the same day) and after a 4-hour fast from food.
3. A complete physical exam (PE) is to be performed at Screening and Study Completion/Early Termination. A symptom-directed PE is to be performed at all other designated visits. Genitourinary exam may be deferred.
4. Patients should be fasting (water only) for a minimum of 2 hours prior to collection of the clinical labs. On dosing days, blood and urine samples should be collected pre-dose.
5. Complete the Early Termination Visit within 30 days of decision to terminate a patient's study participation. Study Completion visit can be performed 84 days after the last study dose (± 14 days).
6. Screening liver biopsy is not required if a patient had a biopsy conducted within one year and a source verifiable medical record specifies no evidence of fibrosis.
7. ECG is optional at Week 28 and Week 52.
8. Negative urine pregnancy test must be confirmed pre-dose on dosing days in pre-menopausal females.

Table 2: Schedule of Assessments – Patients With Evidence of Fibrosis

Assessment	Screen		Day 1 (Dosing Day)	24 hrs After Dosing Day 1	Week 2	Week 4	24 hrs After Dosing Day 29	Week 6	Week 16	24 hrs After Dosing Day 113	Continue q12week dosing until last patient with fibrosis receives 10th dose (Week 100)	Additional Dosing Visits Every 12 Weeks: Weeks 28, 40, 52, 64, 76, 88, 100, 112, 124, 136, 148, 160, 172, 184, and 196	24 hrs After Dosing	Post-Dose Liver Biopsy [5] *See footnote 5 for timing*	90 Days Post Last Dose	Study Completion [6]	Early Termination [8]
	Required Prior to Day 1				Day 15	Day 29 (Dosing Day)		Day 43	Day 113 (Dosing Day)								
Windows (in days)	-60 to -1	-30 to -1		+1	± 5	± 5	+1	± 5	± 14	+1		± 14	+1	± 14	± 5		
Informed Consent	X																
Eligibility Criteria	X																
Demographics/Medical History	X																
PiZZ Genotype	X																
Height, Weight, BMI	X																
Weight only			X			X			X			X				X	X
Urine Cotinine	X								Pre-dose X								
Urine Drug Screen	X																
Hepatitis/HIV	X																
PFT (Spirometry and DLCO) [1]		X	Pre-dose X			Pre-dose X			Pre-dose X			Pre-dose X				X	X
FibroScan	X													X			X
MRI (PDFF, Fe, MRE) [2]	X													X			X
Liver Biopsy [5]	X													X			X
Vital Signs (BP, temperature, HR, RR)	X		Pre-dose & 1 hr post-dose X	X	X	Pre-dose & 1 hr post-dose X	X	X	Pre-dose & 1 hr post-dose X	X		Pre-dose & 1 hr post-dose X		X		X	X
Physical Exam [3]	X		Pre-dose X	X	X	Pre-dose X	X	X	Pre-dose X	X		Pre-dose X				X	X

Table 2: Schedule of Assessments – Patients With Evidence of Fibrosis (Continued)

Assessment	Screen		Day 1 (Dosing Day)	24 hrs After Dosing Day 1	Week 2	Week 4	24 hrs After Dosing Day 29	Week 6	Week 16	24 hrs After Dosing Day 113	Continue q12week dosing until last patient with fibrosis receives 10th dose (Week 100)	Additional Dosing Visits	24 hrs After Dosing	Post-Dose Liver Biopsy [5]	90 Days Post Last Dose	Study Completion [6]	Early Termination [8]
	Required Prior to Day 1				Day 15	Day 29 (Dosing Day)		Day 43	Day 113 (Dosing Day)			Every 12 weeks: Weeks 28, 40, 52, 64, 76, 88, 100, 112, 124, 136, 148, 160, 172, 184, and 196		*See footnote 5 for timing*			
Windows (in days)	-60 to -1	-30 to -1		+1	± 5	± 5	+1	± 5	± 14	+1		± 14	+1	± 14	± 5		
ECG [7]	X		Pre-dose X			Pre-dose X			Pre-dose X			Pre-dose X [7]				X	X
Urine Pregnancy Test [9]	X		Pre-dose X			Pre-dose X			Pre-dose X			Pre-dose X				X	X
FSH (post-menopausal females)	X																
Clinical Labs (heme, coag, trop, chem, lipids, urinalysis) [4]	X		Pre-dose X	X	X	Pre-dose X	X	X	Pre-dose X	X		Pre-dose X		X		X	X
PRO-C3/PRO-C6	X								X			Pre-dose X		X		X	X
Calculate APRI			Pre-dose X						Pre-dose X			Pre-dose X (Weeks 40, 64, 88, 112, 136, and 160)		X		X	X
Anti-drug Antibodies	X		Pre-dose X			Pre-dose X			Pre-dose X			Pre-dose X				X	X
Z-AAT Level	X		Pre-dose X		X	Pre-dose X		X	Pre-dose X			Pre-dose X		X		X	X
Serum Alpha-1 Antitrypsin Level	X		Pre-dose X		X	Pre-dose X		X	Pre-dose X			Pre-dose X		X		X	X
Concomitant Meds/Therapies	X		X	X	X	X	X	X	X	X		X		X		X	X
Adverse Events	X		X	X	X	X	X	X	X	X		X		X		X	X
Study Treatment			X			X			X			X					
Pharmacokinetics (PK)			Pre-dose, 1 hr, & 2 hr post-dose X	X					Pre-dose, 1 hr, & 2 hr post-dose X	X							
Phone Call to Assess AEs													X				
Phone Call to Assess for Pregnancy Post-dose															X		

1. Pulmonary function testing includes: Spirometry including VC, FVC, FEV_1, FEV_1/VC, FEV_1/FVC, and DLCO. Pertinent study FEV_1 will be based on post-bronchodilation value. Spirometry testing at Screening is to determine inclusion/exclusion. Spirometry and DLCO may be repeated once per timepoint. Spirometry and DLCO should also be conducted any time a patient experiences a COPD exacerbation. For dosing visits between Week 28 and Week 196, DLCO can occur once every 6 months.
2. MRI hepatoscan will include magnetic resonance elastography (MRE), hepatic fat content by MRI-PDFF (or equivalent) and hepatic iron content. MRI should always be completed before biopsy (if performed on the same day) and after a 4-hour fast from food.
3. A complete physical exam (PE) is to be performed at Screening and Study Completion/Early Termination. A symptom-directed PE is to be performed at all other designated visits. Genitourinary exam may be deferred.
4. Patients should be fasting (water only) for a minimum of 2 hours prior to collection of the clinical labs.
5. A post-dose biopsy visit will occur at Week 48 (± 2 weeks) for patients with evidence of fibrosis at Screening. For patients beyond Week 48 at the time of IRB/EC approval of Protocol v4.0, the post-dose liver biopsy will be performed at Week 72 (± 4 weeks) or Week 96 (± 4 weeks).
6. The Study Completion visit can occur 4 to 12 weeks from the last study dose.
7. For dosing visit between Week 28 and Week 196, ECG can be performed once every 6 months.
8. Complete the Early Termination Visit within 30 days of decision to terminate a patient's study participation. Liver biopsy does not need to be repeated in an Early Termination patient if a post-dose biopsy was performed at Week 48, 72, or 96.
9. Negative urine pregnancy test must be confirmed pre-dose on dosing days in pre-menopausal females.

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2. STUDY INFORMATION AND SIGNATURES

Investigator's Statement:

I have read and understood the information in this protocol and agree to conduct the trial according to the protocol (subject to any amendments) and in accordance with the principles of Good Clinical Practice. I have read and agree to comply with the Investigator obligations stated in this protocol. Any changes in procedure will only be made if necessary to protect the safety, rights, or welfare of patients.

I agree to conduct in person or to supervise the trial.

I agree to ensure that all that assist me in the conduct of the study are aware of their obligations.

Principal Investigator:

_____ _____

Signature Date

_____

Printed Name

3. LIST OF ABBREVIATIONS AND TERMS

AAT	Alpha-1 antitrypsin
AATD	Alpha-1 antitrypsin deficiency
ADA	Anti-drug antibody
ADS-001	Drug substance (API, lyophilized powder) containing RNAi trigger (also referred to as fazirsiran, ARO-AAT, or TAK-999)
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
API	Active Pharmaceutical Ingredient
APRI	Aspartate aminotransferase-to-platelet ratio index
ARC-AAT	Arrowhead previous clinical stage RNAi therapeutic candidate for AATD; discontinued
ARO	Arrowhead Pharmaceuticals, Inc
ARO-AAT	Short name for ARO-AAT Injection
ARO-AAT Injection	Clinical drug product solution ready for SC injection (also referred to as Fazirsiran Injection or TAK-999 Injection)
AST	Aspartate aminotransferase
BMI	Body mass index
BP	Blood pressure
cGCP	Current Good Clinical Practice
cGMP	Current Good Manufacturing Practice
COPD	Chronic obstructive pulmonary disease
CRA	Clinical Research Associate
CRO	Contract Research Organization
CS	Clinically significant
CTN	Clinical Trial Notification
CV	Coefficient of variation
CVA	Cerebrovascular accident
DLCO	Diffusing capacity for carbon monoxide
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eGFR	Estimated glomerular filtration rate
EOS	End of Study
FAS	Full Analysis Set
Fazirsiran	Drug substance (API, lyophilized powder) containing RNAi trigger (also referred to as ADS-001, ARO-AAT, or TAK-999)
Fazirsiran Injection	Clinical drug product solution ready for subcutaneous injection (also referred to as ARO-AAT Injection or TAK-999 Injection)
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in one second

FEV_1/FVC	Ratio of the forced expiratory volume in the first one second to the forced vital capacity of the lungs
FEV_1/VC	Ratio of the forced expiratory volume in 1 second to vital capacity
FIB-4	Fibrosis-4 index
FSH	Follicle-stimulating hormone
FVC	Forced vital capacity
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
GLP	Good Laboratory Practice
GOLD	Global Initiative for Chronic Obstructive Lung Disease
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDL	High-density lipoprotein
HIV	Human immunodeficiency virus
HR	Heart rate
ICH	International Council for Harmonisation
INR	International normalized ratio
IRB	Institutional Review Board
ISR	Injection site reaction
IUD	Intrauterine device
IWRS	Interactive Web Response System
LD	Lactate dehydrogenase
LDL	Low-density lipoprotein
MAR	Missing at random
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MCV	Mean cell volume
MI	Multiple imputation
mmHg	Millimeters of mercury
MNAR	Missing Not at Random
MRE	Magnetic resonance elastography
MRI	Magnetic resonance imaging
MRI-PDFF	Magnetic resonance imaging proton density fat fraction
mRNA	Messenger RNA
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NHP	Non-human primate
NOAEL	No-observed-adverse-effect level
NSAID	Nonsteroidal anti-inflammatory drug
PBO	Placebo
PD	Pharmacodynamic
PFT	Pulmonary function test
PI	Principal Investigator
PiZZ	Homozygous Z allele individuals

PK	Pharmacokinetic(s)
PT	Preferred Term
PTT	Partial thromboplastin time
Q28	Once every 28 days
Q4W	Once every 4 weeks
QT	QT interval - a measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle
RBC	Red blood cell
RNAi	RNA interference
RR	Respiratory rate
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard deviation
siRNA	Small interfering RNA oligonucleotides
SOA	Schedule of Assessments
TIA	Transient ischemic attack
ULN	Upper limit of normal
VC	Vital capacity
WOCBP	Women of childbearing potential
Z-AAT	Mutant AAT protein from Z allele

4. INTRODUCTION

4.1. Background Information

Alpha-1 antitrypsin deficiency is an autosomal co-dominant genetic disorder with a prevalence range of 1/1500-1/5000 that causes early pulmonary disease in adults and liver disease in children and adults (Nelson et al., 2012). Alpha-1-antitrypsin (AAT) is a 52 kDa circulating glycoprotein protease inhibitor of the serpin family. The primary function of AAT is to inhibit neutrophil elastase to prevent excessive elastase-induced tissue damage. Normally, AAT is synthesized primarily in hepatocytes and several grams daily are secreted directly into the serum. In lung parenchyma, AAT is critical for protection of alveolar interstitial elastin from degradation by neutrophil elastase. A lack of adequate levels of functional AAT leads to damage of lung elastin by neutrophil elastase and the development of early emphysema. It generally takes decades for lung disease to manifest and usually requires additional environmental insult, usually cigarette smoking. Low plasma AAT levels that lead to pulmonary disease in individuals homozygous for the Z mutation (PiZZ) are not from a lack of synthesis (except in null/null patients) but from a disruption of its processing and secretion by hepatocytes. AAT is normally secreted in monomeric form, but the mutant AAT protein from Z allele (Z-AAT) synthesized by PiZZ individuals contains a single point mutation that results in low secretion, polymer formation and accumulation in hepatocytes leading to liver disease. Lung disease is frequently treated with AAT replacement therapy, and fewer than 10,000 patients are on replacement or "augmentation" therapy in the U.S. (Stoller et al., 2012). However, augmentation therapy does nothing to treat liver disease, and no specific therapy is available for alpha-1 antitrypsin deficiency (AATD)-associated liver disease.

In clinical practice, over 90% of AAT deficiency is due to the PiZZ genotype (De Serres et al., 2012). PiZZ adult patients may initially present with clinical signs of pulmonary disease such as dyspnea, cough, or chronic bronchitis, or they may initially present with signs of liver disease such as elevated transaminases or bilirubin, hepatitis, or cirrhosis (American Thoracic Society/European Respiratory Society 2003). Pediatric patients typically present with clinical symptoms of liver disease, which may include asymptomatic chronic hepatitis, failure to thrive, poor feeding, or hepatomegaly and splenomegaly. However, disease natural history in both pediatric and adult patients is variable.

A 2018 publication by Clark et al., examined 94 PiZZ adults using liver biopsy and various other noninvasive measures of liver disease (e.g., transient elastography, Fibrosis-4 index [FIB-4]) (Clark et al., 2018). In this cohort, the prevalence of clinically significant liver disease (≥F2) was 35.1%. The presence of accumulated Z-AAT globules, portal inflammation, and hepatocellular degeneration were associated with clinically significant fibrosis. Similarly, accumulation of Z-AAT globules, portal inflammation, and hepatocellular degeneration are seen on histologic evaluation of the PiZ mouse model liver.

4.2. Development and Mechanism of Action of Fazirsiran

Since Z-AAT protein accumulation is the underlying cause of hepatocyte injury in AATD, preventing Z-AAT synthesis is a rational step to prevent hepatic Z-AAT polymerization,

aggregate formation, and accumulation, and thus a logical approach for treating AATD-associated liver disease. This is supported by the lack of liver disease in AATD patients with null/null genotypes. These rare patients completely lack AAT synthesis. They present clinically with pulmonary disease, but since they have no hepatocyte production or accumulation of mutant AAT protein, they are devoid of liver disease.

One mechanism of preventing Z-AAT accumulation is through RNA interference (RNAi)-mediated gene silencing of Z-AAT protein production. RNA interference (RNAi)-based therapeutics have the potential to silence the expression of any disease gene. RNAi is a naturally occurring process by which small interfering RNA oligonucleotides (siRNAs) trigger a sequence-specific down-modulation of gene expression. By delivering siRNAs targeting AAT sequences to the liver, it is possible to knock down expression of AAT messenger RNAs (mRNAs) in hepatocytes. This reduces the synthesis of Z-AAT proteins that are responsible for hepatic disease in AATD. Reductions in levels of Z-AAT protein production should allow for degradation of Z-AAT proteins already present, prevent further hepatocyte injury, reduce inflammation and fibrosis, and allow for hepatic healing. Knocking down Z-AAT protein production with resulting reduction of both mutant protein aggregates and fibrosis in the PiZZ transgenic mouse has been well demonstrated (Teckman et al., 2013). Based on findings in mouse models of AATD-associated liver disease and effective treatment of other hepatic diseases such as viral hepatitis, it is also expected that with effective reduction in necro-inflammation, fibrosis and early cirrhosis should reverse, as well, with effective, chronic treatment.

Arrowhead Pharmaceuticals, Inc. has developed a drug candidate, fazirsiran (TAK-999, also referred to as ARO-AAT) to treat AATD-associated liver disease through an RNAi-mediated mechanism. Fazirsiran is a novel hepatocyte targeted RNAi trigger molecule which is conjugated to N-acetyl-galactosamine to facilitate hepatocyte endocytosis. Fazirsiran is highly effective at knocking down the AAT mRNA gene transcript and reducing the production of hepatic Z-AAT protein.

4.3. Fazirsiran Preclinical Pharmacology and Studies

Preclinical pharmacology of fazirsiran was evaluated in the PiZ transgenic mouse model of AATD liver disease and in cynomolgus monkeys. In the mouse model, treatment with fazirsiran resulted in dramatically reduced serum Z-AAT protein levels, which correlated with reduced liver Z-AAT mRNA levels. Multi-dose studies showed that mice treated with fazirsiran have less Z-AAT burden in their liver compared to saline-treated mice. Liver Z-AAT burden is determined by separating a liver homogenate into a soluble and insoluble fraction; the insoluble fraction represents aggregates of polymerized protein. As expected, these mice also showed reductions in hepatocyte necrosis, liver inflammation, and down-regulation of fibrosis-related genes. Importantly, hepatocyte necrosis and liver inflammation are also seen on histologic evaluation of human PiZZ patient livers. Fazirsiran treatment in cynomolgus monkeys resulted in a maximum reduction in circulating AAT of approximately 93% with 80% or greater knockdown sustained for more than 8 weeks after receiving two once every 4 weeks (Q4W) 3 mg/kg doses of fazirsiran. Further information on the preclinical pharmacology studies is provided in the Investigator's Brochure.

4.4. Fazirsiran Preclinical Pharmacokinetic and Product Metabolism Studies

Pharmacokinetic (PK) parameters for fazirsiran have been evaluated in both rats and monkeys. Results of these studies can be found in the Investigator's Brochure.

4.5. Fazirsiran Preclinical Toxicology Studies

Fazirsiran has been clinically well tolerated in rats and in non-human primate (NHP) toxicology studies. Details regarding Good Laboratory Practice (GLP) and non-GLP toxicology results are provided in the Investigator's Brochure.

4.6. Fazirsiran Clinical Pharmacology, Pharmacokinetic and Clinical Safety

Fazirsiran has been evaluated in the AROAAT1001 study which investigated the safety and pharmacodynamic (PD) effects of single and multiple doses of fazirsiran in a healthy volunteer population. Results of the AROAAT1001 study are available in the Investigator's Brochure. Fazirsiran is also being evaluated in an ongoing, open-label pilot Phase 2 study in patients with AATD liver disease (AROAAT2002 study). Results of an Interim Analysis of the AROAAT2002 study are available in the Investigator's Brochure.

4.7. Rationale for the Study

Intracellular mutant (Z-AAT) misfolded protein accumulation is the underlying cause of hepatocyte injury in AATD (Torres-Duran et al., 2018). Preventing hepatic accumulation of misfolded protein by silencing Z-AAT synthesis is a logical intervention for treating AATD associated liver disease. This is supported by the development of liver tumors and upregulation of fibrosis associated genes in the PiZ transgenic mouse model and by the lack of liver disease in AATD patients with null/null genotypes versus the presence of liver disease in PiZZ genotype patients (Feldman et al., 1975). This is supported by improvement in liver histology, reduced liver Z-AAT protein (soluble and insoluble aggregates) and reduced fibrosis associated gene expression seen in PiZ mice treated with siRNA targeting the *SERPINA1* gene product (Wooddell et al., 2016). Treatment of patients with fazirsiran is expected to reduce hepatic production of Z-AAT, leading to reductions in intrahepatic misfolded soluble and insoluble aggregated Z-AAT protein and reduction in serum AAT levels. Data obtained from nonclinical pharmacology studies in the PiZ mouse model have demonstrated that multiple doses of fazirsiran administered every other week for 4 doses can prevent the accumulation of, and even reduce the burden of already present Z-AAT protein in the livers of PiZ mice (Wooddell et al., 2020). Based on this animal data, it is expected that multi-dose treatment with fazirsiran in AATD patients will prevent further accumulation of intrahepatic mutant protein while allowing endogenous clearance mechanisms to remove Z-AAT protein already accumulated. This removal of the offending agent should reduce/eliminate inflammation, which should prevent further liver injury while allowing fibrosis due to earlier injury to remodel. The ability of the liver to heal with removal of an inciting insult has been demonstrated with treatment of other causes of liver

injury including hepatitis B virus (HBV), hepatitis C virus (HCV), and nonalcoholic fatty liver disease (NAFLD)/ nonalcoholic steatohepatitis (NASH) (Ellis et al., 2012).

The AROAAT2001 design and conduct is supported by existing nonclinical pharmacology and toxicology data as well as data from a Phase 1 clinical study AROAAT1001 in healthy volunteers which evaluated safety, PK, and PD effect of single and multiple escalating doses of fazirsiran. AATD patients with AATD associated liver disease will require multiple doses of fazirsiran to sustain hepatic silencing of AAT production. Accordingly, this study uses multiple doses of fazirsiran with each dose after the second dose administered approximately every 3 months.

Three different dose levels are being tested. The dosing regimen utilized in this study is supported by the PD effect and safety profile of three once every 28 days (Q28) doses of fazirsiran, which was evaluated in the AROAAT1001 study. Single dose PD effect from the AROAAT1001 study indicates that AAT serum protein levels generally reach nadir, but not always full suppression to below lower limit of quantitation around Week 6 to 8 and start to rebound by approximately Week 12 after a single dose. This observed time until rebound supports quarterly dosing. This study also demonstrated that a second dose at Day 29 (4 weeks after initial dose) resulted in complete suppression of circulating AAT levels that was sustained. (See Investigator's Brochure for details.) Based on these results, it was determined that 2 doses administered 28 days apart would quickly lead to maximal and sustained reduction in hepatic AAT production but thereafter dosing could be quarterly. Hence, this was viewed as the best approach to achieve the fullest level of hepatic reduction of the insulting agent while minimizing overall patient exposure by moving to quarterly dosing.

Based on non-clinical studies in the PiZ mouse model and experience with liver recovery from other necro-inflammatory diseases such as viral hepatitis, it is highly likely that dose administration beyond 3 doses will be required to reduce insoluble aggregated liver Z-AAT sufficiently to reduce globule inflammation and hopefully allow repair of hepatic architecture. Based on other precedents as discussed above, improvement of fibrosis or other adverse liver histologic findings due to AATD liver disease may require long-term therapy to show improvement.

The current study uses liver biopsy to obtain tissue samples to directly measure drug effect on hepatic Z-AAT protein and histopathology. Noninvasive measures (including serum biomarkers, FibroScan®, and magnetic resonance imaging [MRI]) of PD effect are also included. However, liver biopsy remains the gold standard for histological evaluation in clinical studies and is required to obtain tissue specimens for analysis of intrahepatic Z-AAT levels.

4.8. Risk Assessment for Patients

Based on the safety review of nonclinical and clinical trials, the following are considered as identified risks or potential risks:

Identified risks:

- **Injection Site AE Risk:** Adverse events (AEs) at the injection site (e.g., erythema, pain, etc.) have been reported with administration of fazirsiran in clinical studies AROAAT1001, AROAAT2001, and AROAAT2002 and are therefore considered an identified risk. All were deemed to be non-serious and resolved without sequelae. The majority of the injection site reactions (ISRs) were mild in severity. Other siRNA antisense oligonucleotides (ASOs) in clinical trials have been associated with usually mild ISRs. This study includes directions for assessing injection site AE intensity based on pre-defined criteria (see Section 10.5.2). Additionally, steps will be taken to minimize ISRs such as rotating injection sites and allowing the fazirsiran solution to come to room temperature prior to injecting.

Potential risks (monitored in this study using diagnostic evaluations, assessments, and study modification rules):

- **Liver Biopsy Risk:** Performance of liver biopsies is commonly part of the standard of care in patients with liver disease related to AATD as well as other adult liver diseases. While it is a common procedure, like any procedure it is associated with some risk. See Appendix 5 for more information about risks associated with study assessments such as liver biopsy.

- **Pulmonary Risk**: AATD may manifest as pulmonary disease in adult patients. AATD pulmonary disease is often treated with AAT augmentation therapy in countries where this therapy has been approved and is available. Fazirsiran is intended to reduce hepatic production of AAT and will, by extension, reduce serum levels of AAT protein. In AATD patients, this protein is poorly functional in its capacity to inhibit neutrophil elastase relative to wild type protein. Pulmonary disease in AATD requires decades to develop, especially in the absence of smoking, and it is extremely unlikely that short-term reduction in serum AAT will worsen pulmonary disease, particularly in non-smokers. The Sponsor anticipates an approximate 90% reduction of serum AAT to represent near maximum reduction possible with a hepatocyte targeted RNAi mechanism based on previous experience. Extra-hepatic production, including locally in the lung, is not expected to be reduced by fazirsiran.

  Based on the lack of a consistent pattern of adverse changes in pulmonary function metrics seen in the AROAAT1001 study using siRNA to transiently reduce serum AAT levels, and the fact that the native Z-AAT has reduced inhibitory effect against neutrophil elastase, the reduction of serum Z-AAT should not significantly impact any protective effect. Pulmonary disease in PiZZ patients with very low serum AATD takes decades to develop. PiZZ patients already have very low serum AAT levels and a further reduction in already low AAT may not influence pulmonary function. Additionally, many of these patients are on AAT augmentation therapy or may be given augmentation therapy and will continue it during the trial. Steps will be taken in this study to mitigate this theoretical risk.

  1. Patients who smoke or with a significant smoking history will be excluded from this study and their non-smoking status will be confirmed by measuring urinary cotinine at Screening and with repeat confirmatory checks during the study. Patients with a positive

urine cotinine during the study (with positive result confirmed on repeat) will be counseled on the importance of not smoking during the study.

2. Patients with severe and unavoidable exposure to inhaled environmental exposure will be excluded.

3. Above standard of care pulmonary monitoring is implemented during the study with vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), ratio of the forced expiratory volume in the first one second to the forced vital capacity of the lungs (FEV_1/FVC), and the ratio of the forced expiratory volume in 1 second to vital capacity (FEV_1/VC), measured pre-dose and at every dosing visit. Diffusing capacity for carbon monoxide (DLCO) is measured every dosing visit through Week 16 and then every other dosing visit.

4. Patients without sufficient pulmonary reserve at baseline (e.g., post-bronchodilation FEV_1 <65 % of predicted) are excluded. Patients with recent lower respiratory infections (such as pneumonia) are also excluded.

5. Standard of care emphysema treatment (e.g., corticosteroids, bronchodilators) is permitted.

6. Any patient developing pulmonary symptoms or worsening pulmonary function tests (PFTs) while on study will be referred to a pulmonologist for consideration of AAT augmentation therapy, which will be provided by the Sponsor if not otherwise available locally and if indicated in the opinion of the treating pulmonologist (See Pulmonary Study Modification Rules in Appendix 3).

7. Arrowhead will review all VC, FVC, FEV_1, and DLCO test results on a weekly basis during Safety Review Team meetings. Pulmonary function test results will be exported directly from the clinical trial database and any resulting decreases >10% from baseline will be identified. A query will be sent to the Investigator to confirm a pulmonary consultation has been requested and further dosing will be suspended for this patient until the consultation has occurred. Arrowhead follow up with sites will occur in order to obtain the pulmonologist evaluation.

8. The AROAAT2001 study will have a Data Safety Monitoring Board (DSMB). The DSMB Members will review all pulmonary function data and, in particular, review the patient data holistically where a FEV_1 >10% decline from baseline occurs. The Data Safety members will review those patients in an unblinded manner when deemed necessary to promptly identify if an imbalance in treatment groups occurs. It is the DSMB's responsibility to make recommendations to Arrowhead regarding the study conduct (including patient discontinuation from the study per protocol) in a manner that preserves the study blind as appropriate.

- **Hepatic Risk:** Fazirsiran targets the hepatic synthesis of AAT. Arrowhead has not seen a pattern of adverse transaminase changes in the AROAAT1001 healthy volunteer single

ascending doses/multiple ascending doses study. Others developing siRNA for AATD have seen evidence of mild to moderate elevations in transaminases using hepatocyte-targeted siRNA conjugates similar to those used by the Sponsor. It has been described that these alanine aminotransferase (ALT) changes were due to off-target effects of the siRNA seed region on microRNAs in the hepatocyte (Vaishnaw et al., 2017; Schlegel et al., 2017). The siRNA sequence of the fazirsiran sense and antisense molecules have been screened for potential mRNA and microRNA homology and sequences with homology were excluded from consideration. Thus, no such off-target effects are anticipated. In chronic GLP toxicity studies in rats and monkeys, adverse liver findings were not identified, with the no-observed-adverse-effect levels (NOAELs) of 120 mg/kg in rats and 180 mg/kg in monkeys, which in both cases were the highest doses tested. Given the very large safety margins based on the chronic GLP toxicity study NOAELs, liver toxicity in humans is not expected. However, to further mitigate this risk of adverse liver findings, the proposed study protocol has built in stopping rules for ALT elevation applicable to patients who may have elevated ALT at baseline. Blood samples will be drawn frequently to evaluate liver injury and liver function. The DSMB will include hepatologists experienced in adjudication of drug induced liver events. Additionally, the planned doses used in this study of 25, 100, and 200 mg are approximately 1/336th, 1/84th, and 1/42nd, respectively (assuming weight-based conversion and a 70-kg subject) of the rat NOAEL of 120 mg/kg and 1/504th, 1/126th, and 1/63rd, respectively, of the 180 mg/kg monkey NOAEL from chronic GLP toxicity studies. Additionally, the doses used in this study are lower than the 300 mg dose level which was administered for 3 Q28 doses in the AROAAT1001 study and was well tolerated.

Additional information about identified and potential risks is provided in the Investigator's Brochure.

4.9. Justification for Dose Levels and Dose Intervals

The proposed dose levels of 25, 100, and 200 mg are each expected to produce PD effect. In the AROAAT1001 study, doses of 35, 100, and 200 mg yielded substantial serum AAT reductions, with both 100 and 200 mg reaching approximately 90% mean serum AAT reduction after multiple doses in the AROAAT1001 study. All proposed doses are below the top dose of 300 mg, which was studied as both single and multiple (3) doses in AROAAT1001. In the Phase 1 study, dose levels up to the top dose of 300 mg were well tolerated with no serious or severe AEs reported in the study to date and no pattern of adverse laboratory changes associated with fazirsiran. All 3 proposed dose levels are expected to provide varied and dose dependent PD effect and are well within a margin of safety based on previous human testing as well as rat and NHP GLP toxicity studies. A bracketing dose design was chosen based on doses and serum AAT activity observed in AROAAT1001 using the lowest dose expected to generate maximal intrahepatic reduction (100 mg) bracketed by one lower dose level (25 mg) and one higher dose level (200 mg). Serum AAT reductions with single and multiple doses of fazirsiran at the 100 and 200 mg dose levels were similar in the AROAAT1001 study with both appearing to generate maximal serum reductions. Single 35 mg doses appear to be less active than 100 mg with regards to serum AAT reduction. It is presently unknown whether AATD patients will exhibit a similar sensitivity to ARO-AAT Injection, i.e., whether near full reduction in serum AAT in healthy volunteers correlates to patient liver reductions. However, data from other siRNA compounds

targeting AAT indicate that PiZZ patients respond to hepatocyte targeted siRNA silencing of AAT in a manner similar to healthy volunteers at equivalent dose levels (Turner et al., 2018). Finally, although similar serum AAT reduction, following multiple doses of 100 and 200 mg, were observed, there is less inter-subject variability in serum AAT reduction at the higher dose level and an apparent longer duration of effect. It is possible that 200 mg will produce greater suppression in liver Z-AAT production than the 100 mg dose. Arrowhead believes the greater the suppression of liver Z-AAT protein production, the greater potential for improvement in disease. This is based on other liver diseases such as HBV/HCV, where full or near full removal of the insulting agent has been observed to allow the liver to begin the regenerative process.

The rationale for the proposed dosing interval is based on the goal of targeting AATD patients exhibiting liver fibrosis with the aim of halting the liver insult and damage produced by polymerized aggregates of Z-AAT as early and effectively as possible. Providing a dose on Day 1 followed by a dose on Day 29 (Week 4) is likely to more rapidly drive hepatic AAT levels to maximally reduced levels (similar to a loading dose strategy) followed by a maintenance dose 12 weeks after the second dose on Day 113 (Week 16) and dosing approximately quarterly thereafter. This strategy of maximizing ablation of liver AAT early should yield the most benefit to patients in terms of liver PiZ protein reduction over the course of the study, while then moving to quarterly dosing should maximize safety by limiting patient exposure to drug. The administration of the third fazirsiran dose (and subsequent doses later in development) at Day 113 (Week 16) and quarterly dosing thereafter is based on AROAAT1001 single dose data showing duration of PD activity with rebound in serum AAT levels beginning approximately 12 weeks after dose administration. Approximate quarterly dosing should minimize additional unnecessary dosing and minimize safety risk.

In chronic GLP toxicity studies in rats and monkeys, adverse findings were not identified, with the NOAELs of 120 mg/kg in rats and 180 mg/kg in monkeys, which in both cases were the highest doses tested. The planned doses used in this study of 25, 100, and 200 mg are approximately 1/336th, 1/84th, and 1/42nd, respectively, (assuming weight-based conversion and a 70-kg subject) of the rat NOAEL of 120 mg/kg and 1/504th, 1/126th, and 1/63rd, respectively, of the 180 mg/kg monkey NOAEL from chronic GLP toxicity studies. Additionally, the doses used in this study are lower than the 300 mg dose level which was administered for 3 Q28 doses in the AROAAT1001 study and was well tolerated.

5. OBJECTIVES

5.1. Primary Objective

To select a single dose for use in later stage development based on a combined evaluation of safety and PD effects of fazirsiran

5.2. Primary Endpoint

- Percent change from baseline at Week 16 in serum Z-AAT

5.3. Secondary Endpoints

- Subject incidence of treatment-emergent AEs
- Absolute and percent change from baseline in total liver Z-AAT (insoluble + soluble) protein at post-dose biopsy visit
- Absolute and percent change from baseline in liver Z-AAT soluble protein at post-dose biopsy visit
- Absolute and percent change from baseline in liver Z-AAT insoluble protein at post-dose biopsy visit
- Absolute and percent change from baseline in liver function tests including ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total bilirubin, direct bilirubin, and international normalized ratio (INR) at Week 16 and over time through End of Study (EOS)
- Absolute and percent change in serum Z-AAT over time through EOS
- Change over time in PK measurements of fazirsiran at timepoints specified in the Schedule of Assessments (SOA)
- Incidence of anti-drug antibodies (ADAs) to fazirsiran
- Change from baseline in Metavir fibrosis stage at post-dose biopsy

5.4. Exploratory Endpoints

- Change from baseline in liver histology (e.g., globules, inflammation, steatosis) at post-dose biopsy
- Change from baseline in hepatic *SERPINA1* mRNA expression at post-dose biopsy
- Change from baseline in liver fibrosis gene expression at post-dose biopsy

- Absolute and percent change from baseline in serum PRO-C3 at Week 16 and over time through EOS

- Change from baseline in noninvasive scoring systems for fibrosis, including aspartate aminotransferase-to-platelet ratio index (APRI) and FIB-4, at Week 16 and over time through EOS

- Change from baseline in FibroScan® over time through EOS

- Change from baseline in hepatic stiffness based on magnetic resonance elastography (MRE) (if available) over time and through EOS

- Change from baseline in hepatic fat content based on MRI (MRI) (MRI-proton density fat fraction [MRI-PDFF] if available) over time and through EOS

- Change from baseline in hepatic iron content based on MRI (if available) over time and through EOS

- Change from baseline in markers of liver fibrosis, globules, and iron content using biomarkers (e.g., PRO-C6) and special stains and imaging (Masson's Trichrome, Sirius Red, Iron, PAS-D), which may include histologic morphometric analysis (if scientifically feasible and sufficient sample available), over time and through EOS

6. STUDY PLAN

6.1. Study Design

A multi-center, multi-dose placebo-controlled Phase 2 study will be conducted to evaluate the safety, efficacy, and tolerability of the investigational product, fazirsiran, administered subcutaneously to patients with AATD.

Study Details

The study will test 3 dose levels compared to placebo. Patients who have signed an Institutional Review Board (IRB)/Ethics Committee (EC) approved informed consent and have met all the protocol eligibility criteria during Screening will be assigned to one of 3 cohorts and randomized 2:1 (active: placebo) within each cohort. The 3 cohorts of the study are as follows:

- **Cohort 1:** 25 mg dose of fazirsiran or placebo
- **Cohort 2:** 100 mg dose of fazirsiran or placebo
- **Cohort 3:** 200 mg dose of fazirsiran or placebo

Within each cohort, requirements for biopsies, dosing schedules, and SOA will be determined based on the patient's fibrosis score during Screening.

Patients With No Evidence of Fibrosis

Patients who have a documented biopsy showing no evidence of fibrosis within 1 year of the Screening visit will not require liver biopsy at any point during the study.

Patients without fibrosis at Screening will receive 2 doses of fazirsiran or placebo on Day 1 and Week 4, as per the SOA. Following their Week 4 dose, these patients remain in the study with regular visits per the SOA until Week 64.

Patients With Evidence of Fibrosis

Patients who have a pre-dose biopsy showing evidence of fibrosis (without definitive cirrhosis) during Screening will have a post-dose biopsy performed at Week 48. If a patient is beyond Week 48 at the time of IRB/EC approval of Protocol v4.0, then the post-dose biopsy will occur at Week 72 or 96. The study will end after the last patient with fibrosis receives their 10^{th} dose (Week 100 visit) or until a long-term extension study is available at the subject's study site, whichever comes first.

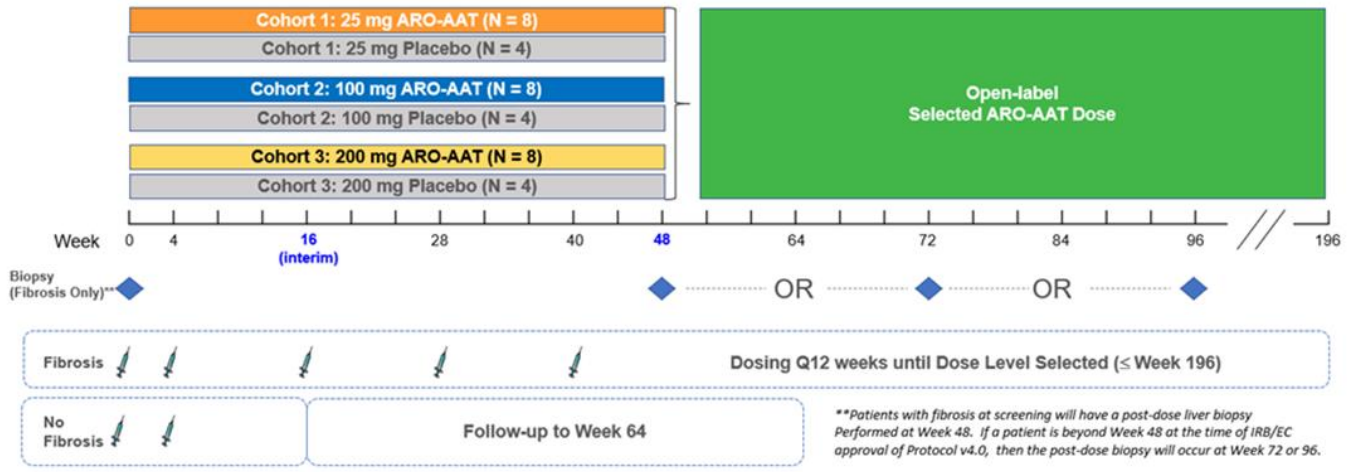
Patients with evidence of fibrosis at Screening will receive a dose on Day 1, Week 4, and Week 16, then every 12 weeks for up to 18 doses total. A Study Completion visit will occur between 4 to 12 weeks after a patient's last study treatment dose.

Dose Evaluation and Selection

After all enrolled patients completed the Week 16 visit, an Interim Analysis was performed to select a single dose level (25, 100, or 200 mg) for the open-label phase of the study. Based on cumulative safety, efficacy, and PD data from the fazirsiran clinical program (clinical studies AROAAT1001, AROAAT2001, and AROAAT2002), a fazirsiran dose (200 mg) was selected

by the Sponsor. All patients with fibrosis at Screening who completed the post-dose biopsy visit at Week 48 (or Week 72 or 96) will receive fazirsiran at the selected dose for the duration of the study (open-label phase) but will remain blinded to their randomized treatment assignment.

Figure 1: Study Schema



6.2. Rationale for Study Design

The study is intended select a dose to be used in later stage development clinical studies and to collect noninvasive markers of liver disease alongside liver biopsy data to demonstrate the relationship of reduction of intrahepatic Z-AAT protein levels to improvement in biomarkers of liver disease. Tissue samples from percutaneous liver biopsy will be required. Other markers of liver injury such as fibrosis score will also be measured on biopsy. Biopsy will be conducted pre-dose and post-dose for patients with evidence of fibrosis at Screening. The study will enroll PiZZ patients without definitive cirrhosis. Patients without evidence of fibrosis will be able to participate in a limited portion of the study. The study uses a placebo arm to help evaluate the safety of fazirsiran in an AATD patient population. The study is double-blind to limit the occurrence of conscious and unconscious bias in trial conduct and interpretation. Blinding will be achieved using a placebo (PBO) (0.9% normal saline) volume matched injection. Inclusion of patients receiving PBO will reduce bias in the assessment of drug safety and tolerability.

In the study, 3 separate dose levels are used to test if there is a dose-response in safety and PD assessments. After all enrolled patients completed the Week 16 visit, an Interim Analysis was performed to facilitate the dose selection. As changes in serum Z-AAT levels in response to fazirsiran is the primary objective of the study, any patient who does not have cirrhosis is eligible to participate. Dose and PD response in patients with cirrhosis will be studied in a separate trial designed for a hepatic impairment population.

Non-cirrhotic PiZZ AATD patients are an appropriate population to conduct dose-finding as serum Z-AAT is readily measured and levels are uninfluenced by fibrosis stage. Thus, all PiZZ patients (without definitive cirrhosis) can participate. Patients without evidence of fibrosis will only receive 2 doses and will not have a post-dose biopsy or continue into the open-label phase as they have no evidence of fibrosis and would be likely to have minimal to no histological

changes following treatment. Therefore, patients with a documented biopsy result showing no evidence of fibrosis within 1 year of screen, will not require a baseline biopsy. In order to maximize the available efficacy data and ensure sufficient samples for analysis of liver biopsies, a minimum of 24 patients with evidence of fibrosis will be enrolled.

Fibrosis patients will have a screening liver biopsy and a second biopsy at Week 48. Changes in liver Z-AAT, adverse liver histological findings and liver injury biomarkers due to AATD liver disease are expected to require 6 months or longer of therapy to show improvement. Thus, a post-dose liver biopsy in patients with evidence of fibrosis at Screening will be taken at Week 48 (if a patient is beyond Week 48 at the time of IRB/EC approval of Protocol v4.0, then the post-dose biopsy will occur at Week 72 or 96).

The open-label phase of the study is intended to collect long-term safety of fazirsiran.

6.3. DSMB and Stopping Rules

An independent DSMB consisting of 2 hepatologists and a pulmonologist and aided by an unblinded biostatistician will review the unblinded data generated from this study after the first 18 patients have received at least one dose of study drug and completed the Week 16 visit, or as needed in emergent safety related circumstances. An independent statistical center (ISC) will provide unblinded comparative safety summaries to the DSMB in a restricted manner. The Sponsor will not have access to the unblinded summaries. This planned safety review will include evaluations for imbalances between active and placebo groups for pulmonary AEs as well as clinically significant adverse changes in FEV_1 and DLCO. The DSMB may be asked to review safety data at additional unscheduled meetings should a potential safety signal be detected. The DSMB will also be involved in decisions to evaluate individual patients for discontinuation (See study modification rules in Appendix 2 and Appendix 3). The DSMB may also make recommendations regarding modification of individual study cohorts and/or study design.

A decision to stop the trial early or discontinue drug in an individual patient or group of patients may be indicated based on any of the following:

1. In the case of 2 or more similar serious adverse events (SAEs) both considered at least possibly related to fazirsiran, the DSMB will meet within 3 days of the DSMB being notified of the second event and within the timeframe of required regulatory agency notification. The DSMB will review available aggregated data to determine if the study remains safe to proceed, should be discontinued, or should continue but with amendments.

2. Evaluation and fazirsiran study modification/discontinuation rules for elevated liver tests signaling worsening AATD liver disease or drug induced liver injury (DILI) are provided in Appendix 2.

3. Evaluation and fazirsiran study modification/discontinuation rules for declines in pulmonary function are provided in Appendix 3.

Any discontinued patient will be followed with appropriate assessments and monitoring (either per SOA or with more intensive evaluation) through EOS. Sponsor or Investigator can discontinue any patient at any time with or without DSMB consultation. If such events (as described in #1 through #3 above) occur and the patient is not discontinued from the study, the reason for not discontinuing the patient will be included in DSMB meeting minutes. Including, but not limited to the events listed above, the DSMB may pause the study to additional dosing to provide time to evaluate safety data and recommend the action to be taken, which may include, but is not limited to, one of the following:

- Discontinuation of a patient or group of patients from the study
- The study is stopped immediately with no further dosing
- The study will continue, but using a lower dose
- The study will continue as planned

Arrowhead will notify the concerned regulatory agencies and ethics committees immediately and at least within 15 days from when the trial is temporarily halted or prematurely discontinued. A substantial protocol amendment will be submitted to the concerned regulatory agencies clearly explaining the reasons and scope, e.g., stopping recruitment and/or interrupting treatment of all patients already included. Furthermore, to restart the trial, the request will be submitted in form of a substantial amendment providing evidence that it is safe to restart the trial. If Arrowhead decides not to recommence a temporarily halted trial, the concerned regulatory agency(-ies) will be notified within 15 days of this decision, providing a brief explanation of the reasons for ending the trial.

6.4. Duration of the Study

The duration of the study is as follows:

Patients without fibrosis: up to 72 weeks, as follows:

Screening: up to 8 weeks

Dosing and Follow-up: up to 64 weeks. Study treatment will be administered on Day 1 and Week 4.

Patients with evidence of fibrosis: up to 216 weeks, as follows:

Screening: up to 8 weeks

Dosing through end of open-label phase: up to 196 weeks, followed by a Study Completion visit 4 to 12 weeks after the last dose in the open-label phase. Up to 18 doses of study treatment will be administered (Day 1, Week 4, Week 16 followed by a dose once every 12 weeks up to Week 196).

7. PATIENT SELECTION

7.1. Number of Patients and Patient Demographics

This study will be conducted in PiZZ patients with confirmed alpha-1 antitrypsin deficiency. Both males and females are eligible, ages 18 to 75 years. In total, the study will consist of approximately 36 patients. A minimum of 24 out of 36 patients must have evidence of fibrosis on the liver biopsy at Screening. All eligible patients will require a pre-dose biopsy completed as part of the study.

7.2. Inclusion Criteria

To be eligible for enrollment, patients must meet all the following inclusion criteria:

1. Male or non-nursing female patients 18 to 75 years of age, inclusive, at the time of Screening with previous diagnosis of PiZZ genotype alpha-1 antitrypsin deficiency. PiZZ diagnosis from source verifiable medical records is permitted. Otherwise, patients must undergo PiZZ confirmatory testing at Screening. PiMZ or PiSZ genotypes are not permitted.

2. Able and willing to provide written informed consent prior to the performance of any study specific procedures.

3. Liver biopsy indicating a liver fibrosis score less than F4 based on local pathologist read.
    a. A patient with no fibrosis may participate based in a previous biopsy conducted within one year if a source verifiable medical record specifies no evidence of fibrosis.
4. A 12-lead electrocardiogram (ECG) at Screening that, in the opinion of the Investigator, has no new acute abnormalities (e.g., new onset atrial fibrillation) that compromise patient's safety in this study. Stable disease (e.g., stable atrial fibrillation) is acceptable.

5. Non-smoker (defined as does not smoke cigarettes daily for at least 12 months) with current non-smoking status confirmed by urine cotinine at Screening and throughout the study AND any previous smoking history prior to 12 months must be <15 pack years. Patients may be on nicotine replacement (patch or gum). E-cigarettes (vapor) are not permitted. A positive urine cotinine result due to nicotine replacement is acceptable for enrollment at the discretion of the Investigator.

6. Use highly effective contraception during the study and for 3 months following the last dose of fazirsiran. Males must not donate sperm for at least 3 months post last dose of study treatment. Women of childbearing potential (WOCBP) must have a negative urine pregnancy test at Screening and on Day 1 pre-dose. Women not of childbearing potential must be post-menopausal (defined as cessation of regular menstrual periods for at least 12 months without an alternative medical cause), confirmed by follicle-stimulating hormone (FSH) consistent with post-menopausal state based on lab reference ranges.

- Using twice the normal protection of birth control by using a condom AND one other form of either birth control pills (The Pill), depot or injectable birth control, intrauterine device (IUD), birth control patch (e.g., Ortho Evra), NuvaRing®, OR Surgical sterilization as a single form of birth control: i.e., tubal ligation, hysterectomy, bilateral oophorectomy, vasectomy, or equivalently effective surgical form of birth control, is acceptable.

- True abstinence for the duration of the study and 12 weeks after the dose of fazirsiran is acceptable only when in line with the preferred and usual lifestyle of the patient. Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea methods are not considered "true" abstinence and are not acceptable methods of contraception.

* All laboratory tests used as inclusion criteria may be repeated once and the repeat value may be used for inclusion purposes.

7.3. Exclusion Criteria

A potential patient will be excluded from the study if any of the following criteria apply:

1. INR ≥1.2 at Screening (one retest permitted). If based on opinion of Investigator and/or prescribing physician patient is appropriate for anticoagulant holiday, patient may stop taking anticoagulant for an appropriate washout period and if indicated a repeat INR within <1.2 would be acceptable. Vitamin K may be used for reversal. If INR is not indicated (direct thrombin inhibitors or Xa inhibitors) then appropriate washout period alone may be acceptable. (Note: Anti-platelet agents, aspirin, clopidogrel or nonsteroidal anti-inflammatory drugs [NSAIDs] are acceptable but must be held 7 days before and 7 days after liver biopsy)

2. Platelet count $<150 \times 10^9$/L at Screening (one retest permitted)

3. ALT and AST levels >250 U/L at Screening (one retest permitted)

4. Estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2 at Screening (one retest permitted)

5. FEV_1 <65% of predicted (preferentially post-bronchodilatory reading) at Screening (one retest permitted)

6. Recent (last 3 months) pneumonia or lower respiratory infection (which must be verifiable from the medical record). Patient reported infection is not sufficient to meet this criterion.

7. Unavoidable exposure to inhaled environmental toxins that in the clinical judgment of the Investigator could impair pulmonary function significantly over the course of the study.

8. Human immunodeficiency virus (HIV) infection, as shown by the presence of anti-HIV antibody (sero-positive)

9. Seropositive for HBV (HBsAg positive at Screening) or HCV (detectable HCV RNA at Screening). Cured HCV (positive antibody test without detectable HCV RNA is acceptable).

10. Uncontrolled hypertension (Systolic BP >170 and diastolic blood pressure [BP] >100 mmHg at Screening). Patients may rescreen once BP is successfully controlled.

11. A history of torsades de pointes, ventricular rhythm disturbances (e.g., ventricular tachycardia or fibrillation), untreated heart block (excluding first-degree block, being PR interval prolongation only), congenital long QT syndrome or new acute ST segment elevation or depression or new acute Q wave on ECG. Stable atrial dysrhythmias (e.g., stable atrial fibrillation) are acceptable.

12. Symptomatic heart failure (per New York Heart Association guidelines), unstable angina, myocardial infarction, severe cardiovascular disease (ejection fraction <20%), transient ischemic attack (TIA) or cerebrovascular accident (CVA) within 6 months prior to Screening

13. History of malignancy within the last 1 year except for adequately treated basal cell carcinoma, squamous cell skin cancer, superficial bladder tumors, or in situ cervical cancer. Patients with other curatively treated malignancies who have no evidence of metastatic disease and >1-year disease-free interval may be entered following approval by the Medical Monitor

14. History of major surgery within the prior 1 month prior to Screening

15. Regular use of alcohol within one month prior to the Screening visit (i.e., more than 14 units of alcohol per week [1 unit = 150 mL of wine, 360 mL of beer, or 45 mL of 40% alcohol])

16. Use of illicit drugs (such as cocaine, phencyclidine [PCP]) within 1 year prior to the Screening visit or positive urine drug screen at Screening (a urine drug screen positive for benzodiazepines, opioids or tetrahydrocannabinol is acceptable for enrollment at the discretion of the Investigator). The patient may still be eligible at discretion of Medical Monitor and Investigator if positive urine drug screen is due to a prescription medication.

17. Use of an investigational agent or device within 30 days prior to dosing or current participation in an investigational study involving a therapeutic intervention. Patients who have participated in the ARCAAT-1001 study or observational studies are acceptable. Patients previously enrolled in but no longer enrolled in gene therapy studies are acceptable. Patients receiving AAT augmentation therapy as part of a post-marketing study or other access program for approved therapies are acceptable.

18. Blood donation (≥500 mL) within 7 days prior to study treatment administration.

19. Any concomitant medical or psychiatric condition or social situation that would make it difficult to comply with protocol requirements or put the patient at additional safety risk. Patients with NASH, NAFLD, metabolic syndrome, well controlled diabetes mellitus (even if on insulin) or hemochromatosis are acceptable if disease is stable and does not pose a significant threat to patient participation. Patients enrolled with NASH should have no plans to undergo bariatric surgery or have initiated or plan to initiate pharmaceutical therapy for NASH (such as Vitamin E or pioglitazone) during the course of the study.

20. A history of thromboembolic disease (including deep vein thrombosis or pulmonary embolism), myocardial infarction, stroke within three (3) months of Screening.

21. Any other condition or finding of clinical relevance at Screening that, in the opinion of the Investigator, would render the patient unsuitable for enrollment or could interfere with participating in and completing the study.

22. Previous diagnosis of definitive liver cirrhosis based on biopsy or complications of cirrhosis (e.g., varices, ascites, hepatic encephalopathy) based on source verifiable medical record.

23. Patients who have undergone lung or liver transplant for AATD are excluded.

Note: Sponsor Medical Monitor has the option to exclude the enrollment of a patient if, based upon the patient's medical history or Screening results, it is felt that a patient's safety may be at risk.

* All laboratory tests used as exclusion criteria may be repeated once, and the repeat value may be used for exclusion purposes.

7.4. Patient Withdrawal Criteria

Patients will be advised that they are free to withdraw from the study at any time for any reason or, if necessary, the Investigator, or medically trained designee, may withdraw a patient from the study, per the following criteria, to protect the patient's health:

- the need to take medication which may interfere with study measurements
- intolerable/unacceptable adverse experiences
- major violation of or deviation from study protocol procedures
- noncompliance of patient with protocol
- patient unwilling to proceed and/or consent is withdrawn or
- withdrawal from the study if, in the Investigator's judgment, it is in the patient's best interest

- pregnancy

The reasons for withdrawal will be recorded on the electronic case report form (eCRF) and included in the final clinical study report, along with any AEs and any necessary medical treatment.

If a patient is withdrawn from the study due to significant AE or SAE, the Investigator, or medically trained designee, will evaluate the urgency of the event. If the situation warrants, the Investigator, or medically trained designee, will take appropriate diagnostic and therapeutic measures. If the situation is not an immediate emergency, the Investigator, or medically trained designee, at the clinical study facility will attempt to contact the study's Medical Monitor or medically qualified designee for consultation. No medical help, diagnosis, or advice will be withheld from the patient due to an inability to contact the Medical Monitor. The patient will be encouraged to remain available for follow-up medical monitoring. The Sponsor will be notified as soon as possible of any patient withdrawals.

Patients who are withdrawn or discontinue prior to EOS visit, will not be replaced.

7.5. Restrictions and Concomitant Medications

1. ***Study Visit Duration:*** For each patient, clinic visits will last approximately 4 hours on dosing days. Patients will return to the clinical facility for outpatient visits as per the SOA. Patients will be observed post-dose for approximately 2 hours or as clinically indicated as per the Investigator.

2. ***Fasting:*** On the day of dosing, patients will fast from food for at least 2 hours prior to study treatment administration. Fasting requirements prior to assessments include:
    a. Blood collection for study labs: patients should fast for a minimum of 2 hours (water only)
    b. MRI/magnetic resonance elastography (MRE): fast for a minimum of 4 hours
    c. Liver Biopsy: fast for a minimum of 6 hours

3. ***Recreational Drugs, Smoking, and Alcohol:*** Patients will be instructed to abstain from consuming alcohol for at least 48 hours prior to their clinic visit on dosing days, and during the clinic visit. In addition, Patients will be instructed to refrain from regular use of alcohol (i.e., more than 14 units of alcohol per week [1 unit = 150 mL of wine, 360 mL of beer, or 45 mL of 40% alcohol]) for the study duration. Patients must abstain from use of recreational drugs throughout the study. Patients must be non-smokers entering the study (as per Inclusion and Exclusion criteria) and abstain from smoking tobacco (including e-cigarettes) for the duration of the study. Nicotine patches or gum is acceptable.

4. ***Concomitant Medications*:** Allowance of concomitant medications will be at the discretion of the study Investigator in consultation with the study's Medical Monitor (when necessary). Augmentation therapy is permitted. Procedural sedation with benzodiazepines or equivalent during MRI or liver biopsy as needed is permitted.

Standard of care for chronic obstructive pulmonary disease (COPD) including bronchodilators, corticosteroids, and other modalities are permitted.

8. INVESTIGATIONAL PRODUCT

8.1. Description, Identification and Dosage

Arrowhead Pharmaceuticals, Inc. is responsible for the supply of Fazirsiran Injection together with detailed instructions (in a Pharmacy Manual) describing preparation of Fazirsiran (TAK-999, ARO-AAT) Injection. The PBO (normal saline 0.9%) will be supplied by the clinical site.

Accordingly, Fazirsiran Injection will be supplied as single sterile 2-mL vial containing the active pharmaceutical ingredient (API) fazirsiran, with the correct dose prepared by the pharmacy or designee prior to dosing patients.

The PBO will be 0.9% normal saline (supplied by the clinical site) administered subcutaneously.

Doses administered per Dose Level:

Each single dose of either active drug (fazirsiran) or PBO (normal saline 0.9%), will be administered by subcutaneous injection. Injections will be made into the subcutaneous tissue at an appropriate site (e.g., abdomen, thigh, upper arm, etc.) using a 25 to 30 gauge, ½ inch needle. The abdomen is the preferred site. The injection site is to be varied (no multiple injections into the same exact site, and alternating various locations on the abdomen is acceptable) and injection site location is to be recorded in the eCRF. Prior to dose administration, the Fazirsiran Injection vial must be allowed sufficient time to come to room temperature. Do not inject into areas of active skin disease or injury such as sunburns, skin rashes, inflammation, or skin infections. Injection volume per injection site should not exceed approximately 1.0 mL. There will be no dose escalation within a cohort (i.e., the same drug dose will be administered to each patient within a cohort). The randomization schedule will be provided to each clinical site and will be maintained along with any other materials that could jeopardize the blind in a secured area of the pharmacy.

8.2. Supply, Preparation, Storage and Labeling of Fazirsiran

Fazirsiran Injection is a ready to use injection preparation for subcutaneous administration and is supplied as a sterile Type-1 glass 2-mL vial (1.1 mL nominal volume, 1.0 mL withdrawable volume) with a fluorocarbon-lined butyl stopper and a red flip-off seal. The clinical drug product is labeled: ARO-AAT Injection.

Strength:	230 mg/mL (salt free basis)
Appearance:	Clear, colorless to yellow solution
Inactive ingredients:	0.5 mM sodium phosphate monobasic, 0.5 mM sodium phosphate dibasic in water for injection
Shipment and Storage:	Refrigerated, 2ºC to 8ºC

The fazirsiran dose will be prepared per the Pharmacy Manual instructions, by a pharmacist or qualified staff at the clinical sites. Aseptic technique will be used throughout dose preparation ensuring sterility of the solution. Because the fazirsiran vial must come to room temperature before administration, the time the vial is removed from the refrigerator and the time of administration must be documented to demonstrate administration within drug stability boundaries.

The investigational product vials will be labeled per current Good Manufacturing Practice (cGMP)/Good Clinical Practice (cGCP).

Fazirsiran Injection will be stored at the clinical site's pharmacy, or other Sponsor-approved storage facility, securely under the appropriate conditions.

8.3. Study Drug Handling

The Sponsor will provide the Investigator with a sufficient quantity of clinical drug supplies. The Investigator must ensure that deliveries of investigational product from the Sponsor are correctly received by unblinded personnel, that all receipts of drug shipments are recorded on the appropriate Drug Accountability forms prepared by the pharmacy at the clinical site and that the products are stored in a secure area under recommended storage conditions. It is also the responsibility of the Investigator to ensure that the integrity of packaged study product is not jeopardized prior to dispensing.

Only patients enrolled in the study may receive study drug, in accordance with all applicable regulatory requirements. Only authorized site staff may supply or administer study drug. The study drug must be stored in a secure area with access limited to authorized unblinded staff and under the physical conditions that are consistent with the study drug-specific requirements.

An authorized and trained staff member at each clinical trial site will dispense the study drug per pre-defined drug dispensing requirements. The dispensing and administration will be verified by a second member of site staff.

Fazirsiran Injection will be supplied by Arrowhead Pharmaceuticals, Inc., and labeled with the drug name, batch number, expiration date (as applicable) and storage conditions. Individual doses will be dispensed by the site pharmacy staff or designee on the day of dosing and recorded in the drug accountability records. A Pharmacy Manual is available to define the procedures for dispensing of study drug.

8.4. Accountability of Study Supplies

All material supplied is for use only in this clinical study and should not be used for any other purpose. The Investigator is responsible for the investigational product accountability, reconciliation and record maintenance at the investigational site. In accordance with all applicable regulatory requirements, the Investigator or designated site staff must maintain investigational product accountability records throughout the course of the study. This person will document the amount of investigational product received from Arrowhead Pharmaceuticals,

Inc., and the amount administered to patients. An unblinded Clinical Research Associate (CRA) will perform initial and ongoing study drug and placebo accountability. The unblinded CRA will protect the integrity of the assignment blind and will not participate in data review for study patients. Used vials of Fazirsiran Injection will be retained and sequestered per patient and cohort (where allowable by local policy) and made available to the unblinded CRA during study drug and placebo reconciliation.

A Drug Dispensing Log must be kept current and will contain the following information:

- the identification of the patient to whom the drug was dispensed; and
- the date(s) and quantity of the drug dispensed to the patient.

The date and time of dose preparation and release will be maintained to support administration of study drug/PBO. The authorized pharmacist or qualified staff will be unblinded to the doses. The pharmacy will dispense the study medication and the study center will administer the study medication only to patients included in this study following the procedures set out in the study protocol and Pharmacy Manual. Each patient will be given only the study medication carrying his/her study number. Study drug administration will be documented on the eCRFs and/or other study drug record. The inventory must be available for inspection by the unblinded CRA during the study. When requested in writing to the Sponsor, following drug accountability and reconciliation, unused drug supplies may be destroyed by the Investigator or designee provided such disposition does not expose humans to risks from the drug and is permitted per the site's Standard Operating Procedures. Records shall be maintained by the Investigator of any such alternate disposition of the test drug. These records must show the identification and quantity of each unit disposed of, the method of destruction (considering the requirements of local law), and the person who disposed of the test drug. Such records must be submitted to the Sponsor.

8.5. Retention of Investigational Product Vials

For this study, used and partially used drug vials will be retained for an adequate period to allow accountability by the unblinded CRA. No additional study drug samples will be retained.

8.6. Allocation to Treatment

All potential patients who sign an informed consent at Screening will receive a unique 6-digit number (i.e., a Screening Number). The first 3 digits will represent the assigned site number and will be the same for each patient that screens at an individual site. The next 3 digits will be assigned sequentially (starting with 001). For patients who are deemed eligible, this 6-digit Screening Number will become the patients permanent study ID number.

Eligible patients will be allocated a unique randomization number, in accordance with the randomization schedule. In the double-blinded phase, each patient will be assigned to either active (fazirsiran) in one of the 3 dose groups or PBO treatment. The allocation of active treatment or PBO will be performed using a block randomization algorithm. In the open-label

phase, ongoing subjects will receive fazirsiran at the selected dose for the duration of the study (see Section 6.1).

Patients who drop out prior to the end-of-study analysis may not be replaced.

8.7. Blinding and Code-break

Blinding of study drug/PBO assignment is critical to the integrity of this clinical trial. It is expected that in most cases, AEs can be properly managed without the need for unblinding. However, in the event of a medical emergency in which knowledge of an individual patient's assignment is considered critical to the patient's well-being and management, the Investigator or documented designated treating physician can unblind the treatment assignment. If the situation is not an immediate emergency, the Investigator should contact the responsible Medical Monitor to discuss the patient and circumstances requiring the unblinding. The blind will be broken only for the specific patient under discussion. The unblinding will be documented in the Electronic Data Capture system. The study monitor should be informed promptly.

The randomization schedules will be maintained under controlled access. The personnel involved in the dispensing of investigational products will be accountable for ensuring compliance to randomization schedules. The non-blinded CRA will review the randomization schedule in comparison to the dispensing log to verify correct randomization.

After all enrolled patients completed the Week 16 visit, an independent statistical center performed the Interim Analysis that facilitated dose selection. Unblinded results of the Interim Analysis were prepared and distributed to the select unblinded Sponsor representatives. Subsequent to the Interim Analysis, the fazirsiran dose for the open-label phase was selected (see Section 6.1). The Sponsor and Contract Research Organization (CRO) study operation team will remain blinded to the individual patient treatment assignment.

Investigators and study patients will remain blinded to the randomized treatment assignment until all patients complete their post-baseline biopsy visit. Following dose selection and Extended Efficacy Analysis (performed after the last patient with fibrosis finishes their post-baseline biopsy visit), Investigators and study patients will be informed of the patient's randomized treatment assignment from the double-blinded phase.

A detailed Data Access Plan outlining which parties will have access to which data and at what time will be prepared. The plan will further indicate which study functions or parties have access to either individual patient or aggregate group treatment information, and/or the selected dose over the duration of the study.

9. STUDY METHODS AND SCHEDULES

9.1. Overview of Procedures

Patients will undergo the following evaluations at regular intervals during the study (refer to the SOA): medical history, physical examinations, vital sign measurements (BP, temperature, heart rate, respiratory rate), weight (at baseline), AE monitoring, ECGs, urine pregnancy test/FSH (females), concurrent medication, pulmonary function testing (spirometry including VC, FVC, FEV_1, FEV_1/VC, FEV_1/FVC, and DLCO) and sample collection for hematology, coagulation, biochemistry, lipids, cardiac troponin, urinalysis, urine cotinine, anti-drug antibodies, drug screens, serum alpha-1 antitrypsin levels (where applicable), serum fibrosis biomarkers, liver biopsy, MRI (where feasible and available), and FibroScan® (where available).

Evaluations during Screening should be completed in the following order (if feasible):

Within Day -60 to Day -1 Screening window: MRI and FibroScan®, followed by liver biopsy

Within Day -30 to Day -1 Screening window: PFTs

Study visits will occur during the Screening window (Days -60 to -1), and as per the SOA. The exception is pulmonary function testing, which must be done within the Day -30 to Day 1 Screening window.

Eligible patients will attend the Clinical Facility on Day 1. Note that study dose administration is on Day 1, which must occur within 60 days of Screening.

On arrival at the clinical facility on Day 1, the Investigator, or designee, will meet with patients to reiterate all study procedures and encourage patients to ask any questions. Patients will fast from food for at least 2 hours pre-dose. Refer to the SOA for additional information. Patients will be confined to the clinical facility for approximately 4 hours on dosing days.

The Investigator (or medically qualified designee) will be required to remain within the clinical study facility for 2 hours after dosing on Day 1 and will remain on call for the duration of the study. Patients should refrain from strenuous physical activities, beyond their normal fitness level, throughout the study.

Clinically significant changes including AEs will be followed until resolution is achieved, or until medically stable or the event is otherwise explained, or until the patient is lost to follow-up. A telephone follow-up will occur 90 days after the last dose to assess for pregnancy occurrence.

9.2. Selection and Screening

Prior to commencement of any Screening procedures, the Investigator, or designee, will inform the patient about the nature and purpose of the study, including the risks and benefits involved, possible AEs, the fact that their participation is voluntary and provide a copy of the IRB/EC approved Informed Consent Form (ICF) for review. Each patient will acknowledge receipt of this information by giving written informed consent for their involvement in the study in the

presence of the Investigator, or designee, who will also sign and date the ICF. Time of consent will be recorded on the ICF or in the source documents for the patient. The original signed consent form will be retained by the Investigator and a copy of the original will be given to the patient. Informed consent will be performed per the Principles of the International Council for Harmonisation (ICH) Good Clinical Practice (GCP) procedures.

Having given Informed Consent, potential patients will undergo procedures outlined in the SOA, to be performed within 60 days of the scheduled dosing date, to determine that they meet the inclusion/exclusion criteria specified in Sections 7.2 and 7.3.

9.3. On-Study Procedures/Assessments

9.3.1. Demographics/Medical History

Medical History will include medication use over the previous 30 days, including vitamins, over-the-counter, prescription drugs, recreational drugs or supplements (such as protein powder or creatine) and alcohol and tobacco use.

9.3.2. Physical Exam

A complete physical exam will be performed at Screening, Study Completion and as per the SOA. At Screening, height (centimetres, without shoes) and weight (kilograms, without shoes) will be obtained to determine body mass index (BMI). At all other timepoints outlined in the SOA, a symptom-directed physical exam will be performed if indicated.

9.3.3. Pulmonary Function Testing

Spirometry and DLCO will be conducted at timepoints outlined in the SOA for all patients. When conducted on dosing days, spirometry and DLCO are to be conducted pre-dose. Spirometry will be conducted in a pulmonology laboratory or at the clinical site in accordance with American Thoracic Society and European Respiratory Society (ATS-ERS) guidelines (Graham et al., 2019) and each patient will undergo pulmonary function testing at the same location throughout the study. DLCO assessment will be conducted in accordance with ATS-ERS guidelines (Graham et al., 2017). All spirometry and DLCO values collected and recorded will include both pre- and post-bronchodilation measurements (unless contraindicated or unavailable).

Spirometry: Spirometry will include VC, FVC, FEV_1, FEV_1/VC, and FEV_1/FVC, all of which will be recorded in the eCRF.

Bronchodilator: At least 3 pre-bronchodilator maneuvers will be performed, followed by administration of a bronchodilator (generally 90 µg albuterol per puff or 100 µg salbutamol per puff), unless contraindicated or unavailable. A site may elect to not perform bronchodilation on certain study subjects if that is in accordance with the site's standard of practice. Following the administration of the bronchodilator, at least 3 post-bronchodilator maneuvers will be performed.

The best pre- and post-bronchodilation measurements should be documented in an eCRF as follows:

- The best FEV_1 is the largest volume FEV_1 from an acceptable maneuver
- The best FVC is the largest volume FVC from an acceptable maneuver
- The best FEV_1/FVC is the best FEV_1 divided by the best FVC, even if these values come from different maneuvers

The number of puffs of bronchodilator administered should be documented, as this information will be captured on the pulmonary function testing eCRF. If administration of bronchodilator during spirometry is not aligned with the institution's practice, the CRA will be notified to ensure Sponsor notification and appropriate study file documentation.

DLCO: The DLCO assessment may be performed either pre- or post-bronchodilation. At least 2 maneuvers should be attempted. The DLCO value recorded in the eCRF should be the average of 2 or more acceptable, repeatable maneuvers.

9.3.4. Electrocardiogram

A single 12-lead ECG measurement will be obtained at timepoints outlined in the SOA after the patient is supine or semi-supine (same position each time is preferred) for at least 3 minutes. Any abnormal ECGs will be repeated in triplicate, with each measurement approximately 1 minute apart. ECGs will be performed prior to venipuncture and other invasive procedures.

9.3.5. Vital Sign Assessments

Systolic/diastolic BP, temperature, HR, and RR (breaths/min) will be obtained at timepoints outlined in the SOA after the patient is seated or semi-supine (same position each time is preferred) for at least 3 minutes. Vitals signs will be obtained prior to venepuncture and other invasive procedures.

9.3.6. Clinical Laboratory Tests

Blood and urine samples will be collected to perform clinical laboratory tests. Patients will be required to fast for at least 2 hours for the Screening sample collections.

At the Screening visit, up to 60 days prior to the first dose of study medication, a fasting blood and urine sample will be collected for the laboratory tests detailed below, to establish baseline data and eligibility for enrolment. The results will be assessed by the Investigator, or medically qualified designee, before study enrolment. Any abnormality in laboratory values (that are confirmed on repeat) deemed clinically significant by the Investigator, or medically qualified designee (i.e., those that would jeopardize the safety of the patient or impact on the validity of the study results), will result in exclusion of that patient. Clinical laboratory tests will be performed on patients' blood and urine at specified time-points listed in the SOA.

***Biochemistry*:** Sodium, potassium, chloride, bicarbonate, glucose, urea, creatinine (including eGFR), creatine kinase, uric acid, phosphate, total calcium, anion gap, cholesterol, albumin, globulins, protein, total bilirubin, direct bilirubin, GGT, ALP, ALT, AST, lactate dehydrogenase (LD), triglycerides, C-reactive protein, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol and Troponin I.

Hematology: Hemoglobin, red blood cell (RBC) count, hematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets, white cell count, neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Coagulation: Partial thromboplastin time (PTT), prothrombin time with INR and Fibrinogen.

Urinalysis: Leukocytes, nitrites, urobilinogen, protein, pH, blood, specific gravity, ketone, bilirubin, and glucose.

Urine Cotinine: urine cotinine levels (at Screening and during study) to confirm non-smoking status.

Microscopic Urinalysis will be Performed if Indicated: White blood cells, RBCs, epithelial cells, bacteria.

Serology: Hepatitis B surface antigen, hepatitis C antibody (with HCV RNA as confirmatory test if needed for positive antibody test) and HIV antibody screen. If necessary, patients will be counseled by the Investigator, or medically trained designee, concerning the blood tests for hepatitis B surface antigen, hepatitis C and HIV antibodies, and their subsequent results.

FSH: Post-menopausal status will be confirmed by FSH level consistent with post-menopausal state.

Drugs Screen: Urine drug screen for benzodiazepines, amphetamines, barbiturates, methamphetamines, methadone, opiates, phencyclidine, cannabinoids, ecstasy, and cocaine.

Pregnancy: Women of childbearing potential will have a urine pregnancy test. If the urine pregnancy test is positive, the patient will not be dosed and will be referred to their primary care provider for follow up.

Anti-drug Antibody (ADA): Serum will be collected to assess for presence of fazirsiran antibodies.

9.3.7. Pharmacokinetics

Plasma samples for analysis of circulating fazirsiran will be obtained at timepoints following study drug administration as outlined in the SOA. Sparse sampling PK blood draws and analysis will be conducted per SOA on all patients enrolled in the study.

9.3.8. Pharmacodynamics

Serum Alpha-1 Antitrypsin Level: Serum levels of alpha-1 antitrypsin will be obtained at Screening and on Day 1 prior to dose administration. Z-AAT serum levels will also be measured as per SOA. The Day 1 value will be used as each patient's baseline value for data analysis purposes. Serum alpha-1 antitrypsin levels will be measured as per the SOA. Samples will be analyzed by standard clinical laboratory assay for serum alpha-1 antitrypsin levels and Z-AAT levels using a Z-specific assay. Z-AAT values will be used for final analysis.

Liver Biopsy: Pre-dose (Screening) and post-dose (per the SOA) percutaneous liver biopsies will be conducted to obtain tissue samples for liver Z-AAT levels and histological evaluation. Per core sample, approximately 30 mm of tissue will be obtained containing at least 11 portal tracts using a 16-gauge needle. Procedure detail and tissue processing protocols are outlined in the Laboratory Manual. Left over tissue samples may be retained and stored for possible future additional analysis. Any retained samples will be deidentified and will not be labeled with patient identifying information. After 10 years from the end of the study, samples will be returned to the primary Institution or local repository or destroyed per local regulations. Tissue samples will be utilized for the following evaluations:

- Soluble liver AAT protein levels
- Insoluble liver AAT protein levels
- Fibrosis gene expression
- *SERPINA1* mRNA expression
- Total (soluble plus insoluble) liver AAT protein levels
- Z-AAT protein PAS/D+ globules (globule size and number)
- Metavir fibrosis stage
- Liver histological evaluation
- Iron content using biomarkers, special stains, and imaging (Masson's Trichrome, Sirius Red, Iron, PAS-D, if scientifically feasible and sufficient sample available)

Two liver biopsy passes will be made to obtain 2 core samples each consisting of approximately 30 mm of tissue. In the event that only one core can be obtained, it should be used for histologic analysis. Additional details around performing biopsy and sample processing/logistics are present in a separate Laboratory Manual.

Serum Fibrosis Markers:

PRO-C3 and PRO-C6 will be measured as a serum marker of liver fibrosis per SOA. Biomarkers of liver disease such as APRI and FIB-4 will be calculated using ALT, AST, age variables.

Magnetic Resonance Imaging (completed if feasible for site): Pre-dose and post-dose (as per the SOA) liver stiffness will be evaluated using MRE. During the same MRI scan, liver fat content via MRI-PDFF (or equivalent methods for determining liver fat) and liver iron content will also be evaluated and quantified. MRE imaging will be conducted per QIBA standards (https://qibawiki.rsna.org/images/9/97/Draft-MRE-QIBAProfile-2017-07-06.pdf). Images will be obtained at the site using a standardized technique. Standardized image processing/interpretation to generate a quantitative MRE value in kilopascals (kPa), % liver fat and iron content will be centralized. Patients must fast from food for at least 4 hours prior to MRI.

FibroScan®: Pre-dose (Screening) and post-dose (as per the SOA) liver stiffness will be evaluated using FibroScan® standard procedures (where available).

9.3.9. Concomitant Medications/Therapies

Patients will be instructed to inform the Investigator of the details (indication, dose, and dates of administration) if they do take any medication, and these details will be recorded in the eCRF. Allowance of concomitant medications will be at the discretion of the study Investigator in consultation with Sponsor Medical Monitor (when necessary). Previous or current use of augmentation therapy is permitted. Sedation with benzodiazepines or alternatives is acceptable during MRI or liver biopsy. Continued use of medications to treat chronic conditions (including COPD) are acceptable.

9.3.10. Follow-Up Procedures: Telephone Call to Assess for Adverse Events

Documented telephone contact with each patient to verify the following:

Adverse Events and Concomitant Medications: 24 to 48 hours after the following doses:

Any dose given after Week 16 (Day 113)

Compliance with contraceptive measures and absence of any known pregnancy:

90 days after the last dose of study treatment

9.3.11. Early Termination Procedures

The reason for Early Termination will be documented in source documents and eCRF. Procedures as outlined in the SOA will be completed. Complete the Early Termination Visit within 30 days of decision to terminate a patient's study participation.

9.4. Allocation of Formulations

In each cohort, and across the study, patients will be randomized in a 2:1 ratio to receive active treatment or placebo in the double-blinded phase. Treatments will be administered per the randomized sequence generated by an Interactive Web Response System (IWRS). Randomizations will occur in parallel across the 3 cohorts.

In the open-label phase, ongoing subjects will receive fazirsiran at the selected dose for the duration of the study (see Section 6.1).

9.5. Study Formulation Administration

Appropriately trained employees of the clinical site will administer the study treatment. There will be no patient self-administration in this study. Each dose will be administered as a single subcutaneous injection. The date, time and location of administration will be recorded in the source notes and witnessed by a second person when administered in the clinical facility. The preferred site of injection is the abdomen. Optional additional sites are the upper arms and thighs. A fazirsiran dose of 200 mg was selected for the open-label phase of the study.

Table 3: Injection Number and Volume per Cohort

Cohort	Dose	Concentration	Total Injection Volume	# Injections per Planned Dose
1	25 mg	230 mg/mL	0.11 mL	Single
2	100 mg	230 mg/mL	0.43 mL	Single
3	200 mg	230 mg/mL	0.87 mL	Single

* Placebo injections of normal saline will be volume matched on a cohort-by-cohort basis.

9.6. Timing of Treatments and Procedures

Actual times of procedures for each patient will vary depending on scheduling and will be recorded in the eCRF.

Post-dose timepoints will be determined from the end of the injection/administration.

In the event of multiple procedures scheduled at the same time, noninvasive procedures (i.e., ECGs, AE assessment) will be conducted prior to invasive procedures (i.e., blood sample collection). Timing of activities may be adjusted slightly to accommodate all procedures.

The following windows are allowed for study assessments/visits:

Pre-dose:	Within three (3) hours prior to dosing
All other procedures through 2 hours:	± 15 minutes
Weeks 2, 4, and 6:	See SOA

Week 16 and beyond: See SOA

9.7. Safety Endpoints

The safety of fazirsiran will be evaluated by collection of the following measurements performed at specified timepoints:

- Monitoring of AEs/SAEs
- Physical examinations
- Vital signs
- ECG measurements
- Clinical laboratory tests (hematology, biochemistry, coagulation, urinalysis)
- Concomitant medications/therapy
- Reasons for treatment or study discontinuation due to toxicity
- Pulmonary Function Testing including spirometry (including VC, FVC, FEV_1, FEV_1/VC, and FEV_1/FVC), and DLCO. Changes in VC, FVC, FEV_1, and DLCO between patients receiving fazirsiran and patients receiving placebo will be analyzed and compared for statistical significance.
- The number of patients receiving fazirsiran versus placebo that require augmentation treatment at any time throughout the study will be summarized.
- Time until initiation of augmentation therapy in active versus placebo patients (Kaplan Meier)
- Number and severity of COPD exacerbations based on Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria in active versus placebo will be evaluated as an AE of special interest.

The AE/SAE reporting period for an enrolled patient will begin when the patient provides informed consent. Treatment-emergent AEs/SAEs will be those defined as following dose administration through EOS or Early Termination. All AEs/SAEs that occur during the AE reporting period specified in the protocol must be reported to Arrowhead Pharmaceuticals, Inc., regardless of the relationship of the AE to study treatment. Any known untoward event that occurs beyond the AE reporting period that the Investigator considers an SAE and possibly related to study treatment will be reported to Arrowhead.

9.8. Sample Processing for Pharmacokinetics

Whole blood will be collected and processed per the Laboratory Manual. Plasma samples will be assayed by a validated hybridization-ligation method. The criteria for repeat analysis, as defined in the respective in-house procedure, will be followed. The validation study conducted by the appointed bioanalytical laboratory to establish validity including accuracy, precision, reproducibility, specificity, recovery, and frozen stability of the analytical method will be appended to the final report.

9.9. Blood Sampling for Pharmacodynamic Analysis

Blood samples will be collected from patients through an indwelling cannula or through a fresh vein puncture. The actual blood collection time will be recorded in the source documents. All deviations outside the range allowed above will be documented as protocol deviations. In all such cases, appropriate time corrections, for the actual time of sample collection will be incorporated at the time of data analysis. Blood samples will be collected at timepoints outlined in the SOA.

The target sample times will be printed in the eCRFs. The actual sample times (times samples taken) will be recorded alongside the nominal times in the eCRF and will be entered at the time of or as soon as possible after sampling. All times must be recorded in the 24-hour format. An explanation must be given for any blood sample taken outside of the set sampling times.

9.9.1. Sample Processing and Analysis for Pharmacodynamic Samples

Pharmacodynamic (AAT): Serum alpha-1 antitrypsin samples will be drawn and analyzed per standard clinical laboratory specifications for serum alpha-1 antitrypsin levels using both a clinical quantitative assay for total AAT and a quantitative specific Z-AAT assay. Left over serum from AAT blood draws will be frozen and used to batch analyze serum Z-AAT levels.

Whole blood will be collected and processed per the Laboratory Manual.

Results, percent change, and duration of response from baseline to timepoints specified in the SOA will be analyzed and summarized by dose cohort and treatment group.

10. ADVERSE EVENTS

The Investigator and clinical facility staff are responsible for detection, recording and reporting of events that meet the criteria and definition of various AEs as listed below. Adverse events will be recorded from time of signed consent through to end of study; only AEs that occur post-dose will be considered treatment-emergent. The Investigator and clinical facility staff are responsible for detection, recording, and reporting of pregnancy and appropriate follow up.

10.1. Definitions

An **Adverse Event (AE)** is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding or diagnostic test), symptom, or disease temporally associated with the use of a medicinal (investigational/experimental) product, whether related to this product or not. (Refer to ICH E2a: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, 27 October 1994).

Treatment-emergent AEs will be defined as AEs with onset after administration of the study drug, or when a pre-existing medical condition increases in severity or frequency after study drug administration.

AEs will not include:

- A medical or surgical procedure such as surgery, endoscopy, tooth extraction, or transfusion (although the condition that leads to the procedure may be an AE)
- A pre-existing disease or condition present at the start of the study that does not worsen during the study
- Any situation where an untoward medical occurrence has not occurred (for example, hospitalizations for cosmetic elective surgery or “social” admissions)
- An overdose of either the investigational product or a concurrent medication without any resulting signs or symptoms

A **Serious Adverse Event (SAE)** is an AE that:

- Results in death
- Is life-threatening, (NOTE: The term ‘life-threatening’ in the definition of ‘serious’ refers to an event/reaction in which the patient was at immediate risk of death at the time of the event/reaction; it does not refer to an event/reaction which hypothetically might have caused death, if it were more severe)
- Requires inpatient hospitalization or prolongation of an existing hospitalization

- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a medically important event or reaction

Medical and scientific judgment should be exercised in deciding whether other situations, should be considered serious such as important medical events that may not be immediately life-threatening or result in death or hospitalization but might jeopardize the patient or might require medical or surgical intervention to prevent one of the other serious outcomes listed in the above definition. These should also be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.2. Clinical Laboratory Abnormalities and Other Abnormal Assessments as AEs

Abnormal assessments (e.g., ECGs and vital signs) that are judged by the Investigator as clinically significant or result in clinical sequelae will be recorded as AEs. Laboratory abnormalities will be reported by the Investigator as AEs if the abnormality is considered clinically significant or result in clinical sequelae. Laboratory abnormalities not reported as AEs are not to be reported as clinically significant (CS) in the study database.

Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs.

The Investigator (or medically qualified designee) will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory result or other abnormal assessment is clinically significant.

10.3. Timing, Frequency, and Method of Detecting AEs

Any pre-existing conditions or signs and/or symptoms present in a patient prior to the start of the study (i.e., before informed consent) should be recorded as Medical/Surgical History.

All AEs occurring after informed consent and on or before the final visit must be reported as AEs; only AEs that occur post-dose will be considered treatment-emergent. All AEs must be recorded irrespective of whether they are considered drug-related.

At each visit/assessment in the period defined above, AEs will be evaluated by the Investigator (or medically qualified designee) and recorded in the medical notes and eCRF.

10.4. Recording of AEs

When an AE occurs, it is the responsibility of the Investigator or medically qualified designee to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event. The Investigator or medically qualified designee will then record the AE on the AE eCRF. Additional reporting requirements for AEs meeting serious criteria are discussed in Section 10.7 below.

The Investigator or medically qualified designee will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In all cases, when available, the diagnosis should be reported as the event and not the individual signs/symptoms. It is not acceptable for the Investigator to send photocopies of the patient's medical records to the Sponsor in lieu of completion of the appropriate AE eCRF pages.

10.5. Evaluating AEs

10.5.1. Assessment of Intensity

The Investigator, or medically qualified designee, will assess intensity (also known as severity) for each AE reported during the study. The assessment will be based on the Investigator's (or medically qualified designee's) clinical judgment. The intensity should be assigned to one of the following categories:

Mild: An event that is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities

Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities

Severe: An event that prevents normal everyday activities

An AE that is assessed as severe should not be confused with a SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 10.1.

10.5.2. Injection Site Reactions

An ISR is defined as an adverse reaction (usually immunologic) developing at the site of study drug injection. AEs at the injection site will be assessed as either Mild, Moderate, or Severe:

- Mild: Tenderness with or without associated symptoms (e.g., warmth, erythema, itching), mild pain or mild edema
- Moderate: Moderate to significant pain or lipodystrophy
- Severe: Tissue ulceration or necrosis with associated severe tissue damage or if operative intervention is indicated

For data analysis purposes, AEs at the injection site with reported terms of bruising or hematoma will not be considered ISRs.

10.5.3. AEs of COPD Exacerbation

AEs reported as an exacerbation of COPD will be assessed as either Mild, Moderate, or Severe according to the GOLD criteria provided below:

Mild: Treated with short acting bronchodilators only

Moderate: Treated with short acting bronchodilators plus antibiotics and/or oral corticosteroids

Severe: Patient requires hospitalization or visits the emergency room. Severe exacerbations may also be associated with acute respiratory failure.

10.5.4. Assessment of Causality

The Investigator (or medically qualified designee) is obligated to assess the relationship between investigational product and the occurrence of each AE. The Investigator (or medically qualified designee) will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the investigational product will be considered and investigated. The Investigator (or medically qualified designee) will also consult the Investigator's Brochure in the determination of his/her assessment.

There may be situations when an SAE has occurred, and the Investigator has minimal information to include in the initial SAE report. However, it is very important that the Investigator (or medically qualified designee) always assess causality for every event prior to transmission of the SAE report form. The Investigator (or medically qualified designee) may change his/her opinion of causality considering follow-up information, amending the SAE report form accordingly. The causality assessment is one of the criteria used when determining global regulatory reporting requirements.

The Investigator (or medically qualified designee) will provide the assessment of causality utilizing 3 possible categories: Not Related, Possibly Related, and Probably Related.

An AE will be considered "Not Related" to the use of the product if any of the following tests are met:

- An unreasonable temporal relationship between administration of the product and the onset of the AE (e.g., the event occurred either before, or too long after administration of the product for it to be considered product-related).
- A causal relationship between the product and the AE is biologically implausible (e.g., death as a passenger in an automobile accident).

- A clearly more likely alternative explanation for the AE is present (e.g., typical adverse reaction to a concomitant drug and/or typical disease-related event).

An AE will be considered "Possibly Related" when there is a reasonable possibility that the incident, experience, or outcome may have been caused by the product under investigation.

An AE will be considered "Probably Related" when there are facts, evidence, or arguments to suggest that the event is related to the product under investigation.

10.6. Follow-up of AEs

After the initial AE, the Investigator is required to proactively follow each patient and provide further information on the patient's condition as deemed appropriate.

All AEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the patient is lost to follow-up. Once resolved, the appropriate AE eCRF page and SAE report form (if event is serious) will be updated. The Investigator, or medically qualified designee, will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. In the event of a fatal outcome in an SAE, the Investigator, or medically qualified designee, will attempt to obtain postmortem findings, including histopathology, and provide all additional information in a follow-up SAE report.

New or updated information regarding an SAE will be recorded on a new SAE report form marked as follow-up with the appropriate follow-up number added to the report. The follow-up report will be signed and dated by the Investigator.

10.7. Prompt Reporting of SAEs

AEs meeting serious criteria MUST be reported promptly to the designated Pharmacovigilance CRO, and the IRB/EC.

10.7.1. Completion and Transmission of the SAE Reports

Once an Investigator becomes aware that an SAE has occurred in a study patient, she/he will report the information on an SAE report form to the designated Pharmacovigilance CRO within 24 hours. The SAE report form will always be completed as thoroughly as possible with all available details of the event and signed by the Investigator (or medically qualified designee). If the Investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before reporting the event. The SAE report form will be updated when additional information is received.

The Investigator (or medically qualified designee) will always provide an assessment of causality at the time of the initial report as described in Section 10.5.4.

Facsimile or email transmission of the SAE report form are the preferred methods to transmit this information to the designated Pharmacovigilance CRO. In rare circumstances, notification by telephone is acceptable, with a copy of the SAE report form sent by overnight mail. Initial notification via the telephone does not replace the need for the Investigator, or medically qualified designee, to complete and sign the SAE report form within the outlined time frames.

The Investigator should report all pregnancies and pregnancies in partners of subjects within 24 hours of awareness of the pregnancy using the Pregnancy Notification Form.

The Sponsor will provide a list of project contacts for SAE and pregnancy report receipt, fax numbers, telephone numbers, and mailing addresses. Any event that in the opinion of the Investigator may be of immediate or potential concern for the patient's health or well-being will be reported to the Sponsor emergency contact listed below.

Sponsor Emergency Contact
[redacted]

10.7.2. Serious Adverse Event Reports to the IRB/EC

The Investigator, or responsible person per local requirements, will comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the IRB/EC.

10.7.3. Pregnancy Reporting

Pregnancy occurring in a subject or in the female partner of a male subject during the study must be reported on the pregnancy reporting form to the designated Pharmacovigilance CRO immediately and not later than 24 hours of initially becoming aware of the pregnancy by the Investigator.

Pregnancy data will be collected at the initial notification, birth/termination of pregnancy, and for up to 1 year after birth or until the end of the pregnancy.

Pregnancies are not SAEs. However, any SAE that occurs during pregnancy (e.g., serious maternal complications, therapeutic or spontaneous abortion, ectopic pregnancy, stillbirth, etc.) must be reported in accordance with the procedure for reporting SAEs.

10.8. Regulatory Requirements for Reporting of SAEs

The Investigator (or medically qualified designee) will promptly report all SAEs in accordance with the procedures detailed in Section 10.7. Prompt notification of SAEs by the Investigator (or medically qualified designee) **is essential** so that the Sponsor may comply with its regulatory obligations.

The Sponsor will comply with all reporting requirements as stipulated in applicable regional/national regulations. The Sponsor is responsible for reporting all suspected unexpected serious adverse reactions to the Eudravigilance database. The Sponsor will ensure that all relevant information about suspected serious unexpected adverse reactions that are fatal or life-threatening is recorded and reported as soon as possible to the competent authorities in all the Member States concerned, and to the EC, and in any case no later than 7 days after knowledge by the Sponsor of such a case, and that relevant follow-up information is subsequently communicated within an additional 8 days. All other suspected serious unexpected adverse reactions will be reported to the competent authorities concerned and to the EC concerned as soon as possible but within a maximum of 15 days of first knowledge by the Sponsor.

10.9. Post-study AEs

A post-study AE is defined as any event that occurs outside of the AE detection period defined in Section 10.3.

Investigators are not obligated to actively seek AEs in former study patients. However, if the Investigator learns of any SAE, including a death, at any time after a patient has been discharged from the study, and he/she considers the event reasonably related to the investigational product, the Investigator will promptly notify Arrowhead.

10.10. SAEs Related to Study Participation

An SAE considered related to study participation (e.g., procedures, invasive tests, a change in existing therapy), even if it occurs during the pre- or post-treatment period, will be reported promptly (refer Section 10.7).

11. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

Statistical analyses and descriptive summaries will be presented for primary, secondary, and exploratory endpoints using appropriate methods. A detailed statistical analysis plan (SAP) will be provided. Descriptive statistics will be presented for all analyses unless otherwise specified. For continuous variables, data will be presented as number (n), mean, median, standard deviation, minimum, and maximum. Discrete variables will be presented as frequencies and proportions or percent. Data will be analyzed by cohort and treatment groups of fazirsiran 25, 100, and 200 mg versus pooled placebo. Data summarized in tables and figures will also be presented in patient listings.

11.1. Study Populations

Four study populations will be defined and evaluated in this study.

- **Safety Population**: All patients who receive at least one dose of study drug. The Safety Population will be used for safety analyses. Patients will be included in the analyses according to the treatment they actually received.

- **Full Analysis Set (FAS):** All randomized patients who receive at least one dose of study drug. The FAS will be used to analyze endpoints related to efficacy. Patients will be included in the analyses according to the treatment planned by randomization.

- **Modified Full Analysis Set (mFAS):** All randomized patients who receive at least one dose of study drug who has pre-dose serum Z-AAT assessment and a Week 16 post-dose assessment.

- **PK population:** All FAS patients who have sufficient plasma concentration data to facilitate determination of PK parameters. Patients with major protocol violations will be assessed on a patient-by- patient basis for inclusion in the PK Analysis set. PK population will be used for PK analyses.

11.2. Sample Size Considerations

Approximately 36 patients will be enrolled and randomized in the study. The primary efficacy analysis based on serum AAT levels, will be executed after all enrolled patients complete Week 16 visit and will evaluate the difference in mean percent change from baseline at Week 16 between each active dose group and the pooled placebo groups. Patients will be randomized in a 2:1 (active:placebo) ratio within each of the 3 dose cohorts. With 8 patients in each of the 3 active treatment dose groups and 12 patients in the pooled placebo group, the study has 99% global power to declare at least one treatment dose group as different from placebo, assuming the treatment difference from placebo of at least 70 percent points and using a one-sided 2.5% level of significance with Hochberg's step-up adjustment for multiplicity of testing. Furthermore, the study has 97% conjunctive power to detect all dose groups that are truly different from placebo and 99% disjunctive power to detect at least one dose group that is truly different from placebo. The estimate of variability of the primary endpoint is calculated from prior studies to be 35%.

11.3. Randomization and Stratification

Following Screening, eligible patients will be randomly allocated in a 2:1 ratio within each dose cohort, to receive one of 3 dose levels of fazirsiran (25, 100, or 200 mg) or placebo. Randomization will be performed within each dose cohort to maintain blinding due to different dose volumes in the double-blinded phase.

Metabolic syndrome has been associated with increased risk of developing liver fibrosis in AATD (Clark et al., 2018). Randomization will be stratified by the presence of metabolic syndrome (defined as in Appendix 1) identified at Screening (Alberti et al., 2009). Randomization will also be stratified by presence of NASH (steatosis, inflammation, and ballooning) at Screening based on pre-dose study biopsy. Patients with both NASH and metabolic syndrome will be stratified based on the presence of NASH.

11.4. Screening Data

Demographics will be tabulated by patient and summarized by cohort and treatment group. Eligibility assessments at baseline, including medical/surgical history data and physical examination data (including height and weight), will be listed for each patient.

11.5. Safety/Tolerability Data

All safety analyses will be performed using the Safety Population. In general, safety analyses will be performed, and the results summarized by cohort and treatment group. All safety summaries will be accompanied by patient listings.

Treatment-emergent AEs will be summarized using the latest version of Medical Dictionary for Regulatory Activities (MedDRA) by System Organ Class (SOC) and Preferred Term (PT), classified from verbatim terms. The incidence and percentage of patients with at least 1 occurrence of a PT will be included, per the most severe grade using a 3-point scale (mild, moderate, severe). The number of events per PT will also be summarized. Causality (relationship to study treatment) will be summarized separately.

The incidence and frequency of AEs, SAEs, treatment-related AEs, treatment-related SAEs, and AEs leading to withdrawal, dose modification, or treatment discontinuation will be summarized by dose and treatment group per SOC and PT. AEs will also be summarized in listings. The duration of AEs will be determined and included in listings, along with the action taken and outcome.

The incidence of laboratory abnormalities will be summarized. Results for variables that are not coded will be presented in the listings as “below, within, and above” the normal limits of the laboratory. Pregnancy test results will be summarized separately by timepoint.

Vital sign measurements will be summarized at each scheduled timepoint using descriptive statistics. Physical examination findings will be summarized by timepoint and presented in patient listings.

ECG parameter changes overall, changes from baseline and qualitative assessments will be summarized.

Change from baseline in VC, FVC, FEV_1, and DLCO will be summarized by timepoint. For FEV_1, summary measures will include change from baseline expressed in mL, as a percentage of baseline value, and in units of percent predicted normal and will also be summarized as upper 95^{th} percentiles with 95% confidence interval (CI). For DLCO summary measures will include change from baseline expressed as absolute change in values and as a percentage of baseline value. Number of patients requiring new initiation of augmentation therapy during the study and time from baseline until new initiation of augmentation therapy (Kaplan Meier analysis) will be evaluated.

11.6. Immunogenicity Data

The number and percentage of patients who are ADA positive at baseline and at any post-baseline visit will be summarized. A listing of patients with positive ADA assay results will be provided.

11.7. Pharmacokinetic Data

The fazirsiran PK concentration data will be listed as appropriate.

The plasma PK concentration will also be used for population PK analysis, combined with PK data from other clinical studies, using nonlinear mixed effect methods. The Population PK analysis will be supported with separate analysis plans and report.

11.8. Pharmacodynamic Data

Serum AAT Levels: The whole blood collected for PD analysis following multiple doses of fazirsiran or placebo will undergo analysis for PiZ protein and total alpha-1 antitrypsin levels using both a Z-AAT specific assay and a clinical assay. Results, including depth of knockdown as reported by percent change from baseline will be analyzed and summarized by dose cohort and treatment group. For data analysis, batched results using nephelometric assay may be used.

MRI and FibroScan®: Noninvasive imaging using both FibroScan® and MRI (evaluation of liver stiffness with MRE, liver fat with MRI-PDFF (or equivalent MR based measure of liver fat) and liver iron content) will be completed pre-dose and post-dose per the SOA in a standardized fashion. Percent change in quantitative measure of liver stiffness, liver fat and liver iron content from pre-dose to post-dose will be analyzed and summarized by dose cohort and treatment group. MRI images will be collected in a standardized fashion with central image processing and analysis to determine quantitative elastography values. It is acknowledged that not all patients will be able to complete FibroScan® or MRI due to availability or individual medical conditions.

Liver Biopsy Assessments: Liver biopsy will be completed pre-dose and post-dose per the SOA or Early Termination. Secondary and exploratory endpoints will be based on comparison of pre-dose biopsy versus post-dose biopsy. The post-dose biopsy may include a biopsy completed at

Early Termination. Biopsy sample will be taken, prepped, and stored in a standardized manner with biopsy Histologic/IHC reads being completed by an independent blinded central pathologist. Percent change in intrahepatic Z-AAT, changes in PAS/D+ globules (size and number), mRNA, changes in Metavir fibrosis scale, fibrosis gene expression levels, and iron content using biomarkers, special stains, and imaging (Masson's Trichrome, Sirius Red, Iron, if scientifically feasible and sufficient sample available) from pre-dose to post-dose sample will be analyzed and summarized by dose cohort and treatment group. A local reader at each site will be used during Screening to determine eligibility based on presence of fibrosis/cirrhosis per inclusion/exclusion criteria.

11.9. Pre-Specified Subgroup Analyses

Separate pre-specified analysis will be performed for the following subgroups.

- Analysis of efficacy, PD, and safety based on pre-dose Metavir fibrosis Score 0-1 versus 2-3.
- Analysis of efficacy, PD, and safety based on the presence of NAFLD or NASH (defined as presence of hepatic steatosis, inflammation and hepatocyte ballooning on histology) versus no NAFLD and NASH.
- Patients on AAT augmentation will be analyzed separately for changes from baseline in serum AAT levels.

11.10. Analysis Methods

The primary objective of the study is to evaluate the 3 doses of fazirsiran versus placebo and select a single active dose to be evaluated further in later stage studies and used in the open-label phase of this study. In addition to examination of safety, dose selection will be based on the evaluation of the mean percent change from baseline (Day 1, pre-dose) in serum Z-AAT levels through Week 16. The primary efficacy analysis, executed after all enrolled patients reach the Week 16 evaluation, will be performed using a MMRM approach. The repeated measures of the response are the values obtained at the scheduled study Weeks 2, 4, 6, and 16. The model will include fixed categorical effects for treatment, time, week, treatment by week interaction, and a baseline value of serum Z-AAT as a continuous covariate. Restricted maximum likelihood estimation (REML) with an unstructured within-patient covariance structure will be used. If the model fails to converge, alternative covariance structures will be considered.

Based on the stated model, least squares (LS) estimates of the mean percent change from baseline for each treatment group, of the difference for each fazirsiran dose group and pooled placebo, and the corresponding 95% CIs will be reported for each timepoint. P-values from the Week 16 2-sided t-tests of each for each of the 3 dose groups vs placebo group will be evaluated for significance using Hochberg's step-up procedure to adjust for multiplicity of testing. Placebo group will be pooled from placebo patients in 3 dose cohorts. The primary evaluation of interest is at Week 16 timepoint, to be completed in all randomized patients who receive at least one dose

of study treatment (FAS), regardless of the total number of received doses or any intercurrent events (hypothetical estimand).

Secondary endpoints include:

- Subject incidence of treatment-emergent AEs
- Absolute and percent change from baseline in total liver Z-AAT (insoluble + soluble) protein at post-dose biopsy
- Absolute and percent change from baseline in liver Z-AAT soluble protein at post-dose biopsy
- Absolute and percent change from baseline in liver Z-AAT insoluble protein at post-dose biopsy
- Absolute and percent change from baseline in liver function tests, including ALT, AST, ALP, GGT, total bilirubin, direct bilirubin and INR at Week 16 and over time through EOS
- Absolute and percent change in serum Z-AAT overtime through EOS
- Change over time in PK measurements of fazirsiran at timepoints specified in the SOA
- Incidence of ADAs
- Change from baseline in Metavir fibrosis stage at post-dose biopsy

If the post-dose liver biopsy is not available, the biopsy completed at Early Termination may be used.

For continuous secondary endpoints, analyses similar to the primary endpoint analysis will be performed. For all continuous longitudinal measures where data is collected at baseline and at more than one pre-scheduled post-baseline visits, a mixed-effects model repeated measures (MMRM) will be applied to evaluate the change from baseline at scheduled visits.

Prior to each analysis of continuous endpoints, the endpoint variable will be evaluated for normality, and as a result, the original data may be transformed to achieve approximate normality. Results of analysis model from transformed data will be appropriately back-transformed to original scale for reporting purpose.

Demographic, PK, PD and safety parameters will be summarized using descriptive statistics (n, mean, SD, % coefficient of variation (CV), geometric mean, geometric %CV, minimum, median, and maximum for continuous parameters; frequency and percentage for categorical parameters).

Sensitivity Analyses of Primary Efficacy Objective

The primary efficacy analysis method, MMRM, is based on the assumption of missing at random (MAR), where patients who discontinue the study prematurely are assumed to have behaved similarly to other patients in the same treatment group had they not dropped out. A sensitivity analysis, performed only if more than 5% of the patients discontinue from the study prematurely, will be performed to evaluate this assumption. The sensitivity analyses will be based on the multiple imputation (MI) control-based imputation method where placebo group is used to impute the missing values of the primary endpoint for the patients in the active treatment group. Specifically, the copy difference in reference method will be used which assumes that patients who withdraw from the active treatment group will have, following the dropout, their efficacy tend toward that of the placebo arm, but starting from the benefit already obtained. Missing endpoint values will be multiply imputed from their approximate posterior predictive distributions, obtained using multivariate imputation by chained equations algorithm. Details of the imputation model will be provided in the SAP. Multiply imputed datasets will be analyzed using the same methods as those in the primary analysis. Results from analysis of each imputed dataset, will be combined using Rubin's combination rule, to produce, if appropriate, a pooled treatment difference and its 95% CI.

A tipping point analysis will also be performed to evaluate the extent of departure from the observed treatment effect in order to arrive at a reversal of study inferences. These analyses will be performed under 2 scenarios regarding the assumptions around missing data mechanism: (1) MAR as applied in the primary analysis using the MMRM model, and (2) Missing Not at Random (MNAR) using the *copy difference* MI as described above.

Exploratory Analyses

The exploratory analyses are designed to investigate the association between treatment with fazirsiran or placebo and various biomarkers. These analyses will be exploratory, and data driven. Additional data, from other clinical trials, are often needed to confirm associations. Some of these exploratory analyses may be reported outside of the clinical study report.

11.11. Interim and Planned Analyses

A single Interim Analysis was performed by the ISC after all enrolled patients completed the Week 16 visit, the timepoint for the primary efficacy evaluation of interest. The unblinded results of the Interim Analysis were prepared for select Sponsor representatives, to facilitate the dose selection. The Sponsor and CRO's study operational team will remain blinded to the individual patient treatment assignment. Investigators and study patients will remain blinded to the randomized treatment assignment until all patients complete their Week 48, post-baseline biopsy visit. Upon completion of the study, Investigators and study patients will be informed of the patient's randomized treatment assignment from the double-blinded phase.

In order to maintain a complete audit trail of activities related to the Interim Analysis and to secure a controlled access to interim results, the study will utilize a controlled access execution system for storage and distribution of Interim Analysis related documents.

A detailed Data Access Plan outlining which parties will have access to which data and at what time will be prepared. The plan will further indicate which study functions or parties have access to either individual patient or aggregate group treatment information, and/or the selected dose over the duration of the study.

The Extended Efficacy Analysis will be performed after the last patient with fibrosis finish post-baseline biopsy visit. The Final Analysis will be performed once all patients complete an Early Termination or Study Completion visit and database lock.

11.12. Data Recording and Quality Control

Source documents must be maintained for each patient in the study, consisting of all demographic and medical information, including clinical laboratory data, etc. A copy of the signed ICF must be retained. All information on the eCRFs must be traceable to these source documents in the patient's file.

Data recorded in all patients' eCRFs will be subjected to a quality control review.

12. STUDY APPROVAL AND CONDUCT

The following conditions will be met.

12.1. Regulatory Approval

The requirements for the conduct of clinical trials in accordance with local applicable regulations will be met before commencement of this study.

12.2. Institutional Review Board (IRB)/Ethics Committee (EC) Approval

Prior to initiation of the study, written IRB/EC approval of the Protocol and ICFs, based on the principles of ICH cGCP procedures, will be received. A copy of the signed and dated letter of approval will be provided to the clinical site and Arrowhead Pharmaceuticals, Inc. prior to study commencement. Any written information and/or advertisements to be used for volunteer recruitment will be approved by the IRB/EC prior to use. A list of the IRB/EC voting members, their titles or occupations, Federalwide Assurance number (where applicable) and their institutional affiliations will be requested before study initiation.

Protocol modifications that may impact patient safety or the validity of the study will be approved by the IRB/EC, following written agreement from the Sponsor.

12.3. Ethical Considerations

This study will be carried out per the Declaration of Helsinki 1964, as modified by the 64th World Medical Assembly, Fortaleza, Brazil, October 2013, the Notes for Guidance on Good Clinical Practice (cGCP) (2000) (CPMP/ICH/135/95), and the Principles of the ICH cGCP. The protocol will be submitted for approval to the IRB/EC, and written approval obtained before patients are enrolled. The composition of the IRB/EC will also be provided to the Sponsor. If approval is suspended or terminated by the IRB/EC, the Investigator will notify the Sponsor immediately.

Where applicable, the clinical site and Arrowhead Pharmaceuticals, Inc. agree to abide by the local compensation guidelines for injury resulting from participating in a company-sponsored research project. Compensation will only be provided on the understanding that the provision of compensation does not amount to an admission of legal liability and is subject to the proposed recipient signing a full and complete release of the company from all claims, damages, and costs.

12.4. Written Informed Consent

Informed consent will be obtained before the patient can participate in the study. The contents and process of obtaining informed consent will be in accordance with all applicable regulatory requirements. Study participation includes all Screening procedures, as well as any washout of excluded medications.

It is the responsibility of the Investigator (or medically qualified designee) to obtain a written informed consent from everyone participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study. The Investigator (or medically qualified designee) must also explain to the patients that they are completely free to refuse to enter the study or to withdraw from it at any time. Appropriate forms for documenting a written consent will be provided by the Investigator or by Arrowhead Pharmaceuticals, Inc.

For this study, each eligible patient will be required to provide written informed consent before participation in the study.

All eligible patients will have the study explained by the Investigator or designee. They will receive a full explanation, in lay terms, of the aims of the study, the discomforts, risks and benefits in taking part as well as of insurance and other procedures for compensation in case of injury. It will be explained that the study is for research purposes only and is not expected to provide any therapeutic benefit to the individual. It will be pointed out that they can withdraw from the study at any time without prejudice. Each patient will acknowledge receipt of this information by giving written informed consent for participation in the study. The volunteer will be given a copy of the signed ICF to retain.

12.5. Emergency Contact with Principal Investigator

Suitable arrangements will be made for patients to contact the Investigator or medically trained designee in the event of an emergency.

12.6. Notification of General Practitioner

It is the responsibility of the Principal Investigator (PI) or designee, to notify, where applicable, with the consent of the patient, the general practitioner of the patient's participation in the trial, by sending a letter stating the nature of the trial, treatments, expected benefits or AEs and concomitant drugs to be avoided.

12.7. Clinical Laboratory Certification and Reference Ranges

Before the initiation of this study, the PI, or designee, will obtain a copy of the certification form, with certification number and expiration date for all clinical laboratories (excluding central laboratories) used in the study. Reference ranges for each clinical laboratory test used in this study will be obtained from the appropriate laboratory, which will perform the test for the study.

12.8. Protocol Deviations

A protocol deviation is defined as any intentional or unintentional change to, or noncompliance with, the approved protocol procedures or requirements. The PI will conduct the study in compliance with the approved protocol and will not implement any deviation from or changes to the protocol without prior agreement by the Sponsor and review and documented approval from the regulatory authorities and IRB/EC of an amendment, except where necessary to eliminate an immediate hazard to study patients.

Deviations may result from the action or inaction of the patient, PI, or site staff. Examples of deviations include, but are not limited to:

- Failure to adhere to study exclusion and inclusion criteria
- Failure to comply with dispensing or dosing requirements
- Use of medications, food, drink, herbal remedies, or supplements that are specifically prohibited in the protocol
- Missed or out-of-window visits
- Drug dosing not administered within the time frame specified in the protocol
- Failure to adhere to test requirements, including vital signs, laboratory tests, physical examinations, PK blood draws, medical history, etc. – either tests not done, incorrect tests done, or not done within the time frame specified in the protocol
- Procedural deviations such as incorrect storage of study drug, failure to update the ICF when new risks become known, failure to obtain IRB/EC approvals for the protocol and ICF revisions

Protocol deviations impacting patient safety or eligibility will be reported to the Sponsor or CRO within two (2) business days of occurrence and to the IRB/EC/competent regulatory authority per local regulatory requirements.

The Investigator is responsible for ensuring that any known protocol deviations are recorded and reported as agreed. The nature and reasons for protocol deviations will be recorded in each patient's eCRF.

12.9. Termination of the Study

The Sponsor reserves the right to discontinue the trial at any time. Reasons will be provided in the event of this happening. The Investigator reserves the right to discontinue the study for safety reasons at any time in collaboration with the Sponsor.

13. STUDY ADMINISTRATION

13.1. Study Monitoring

Arrowhead Pharmaceuticals, Inc. is responsible for assuring the proper conduct of the study about protocol adherence and validity of the data recorded in the eCRFs. Patient confidentiality will be maintained.

In accordance with applicable regulations, cGCP, and Arrowhead Pharmaceuticals, Inc. procedures, Arrowhead Pharmaceuticals, Inc. will be responsible for assigning a study monitor (CRA) who will contact the site to organize a visit prior to patient enrolment to review the protocol and data collection procedures with site staff. In addition, the assigned study monitor will periodically contact the site, including conducting on-site visits. The extent, nature and frequency of on-site visits will be based on such considerations as the study objective and/or endpoints, the purpose of the study, study design complexity, and enrollment rate.

During these site visits, the study monitor will:

- Check the progress of the study
- Review study data collected
- Conduct source document verification
- Identify any issues and address their resolution
- Check investigational product accountability
- Review blood and urine samples and ensure they are labeled and stored correctly

This will be done to verify that the:

- Data are authentic, accurate and complete
- Safety and rights of patients are being protected
- Study is conducted in accordance with the currently approved protocol (and any amendments), cGCP and all applicable regulatory requirements

The PI agrees to allow the monitor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the monitor to discuss findings and any relevant issues.

At study closure, a study monitor will conduct the following activities in conjunction with the PI or site staff as appropriate:

- Return of all study data to Arrowhead Pharmaceuticals, Inc.

- Data queries
- Accountability, reconciliation, and arrangements for unused investigational product(s)
- Inventory and final disposition (e.g., destruction, shipping to repository, etc.)
- Review of site study records for completeness

Because the study is blinded, an unblinded study monitor will be assigned to visit the site pharmacy during, and at Study Completion to review the randomization schedule in comparison to the dispensing log to verify correct randomization of study drug.

13.2. Quality Assurance

To ensure compliance with cGCP and all applicable regulatory requirements, Arrowhead Pharmaceuticals, Inc. may conduct a quality assurance audit of the study site. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the PI and clinical site agree to notify Sponsor as soon as possible following awareness of an impending regulatory inspection. The PI and clinical site agree to allow the auditor/inspector direct access to all relevant documents and allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

13.3. Records Retention

Following closure of the study, the PI must maintain all site study records in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g., audit or inspection) and whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems and staff. When permitted by local laws/regulations or institutional policy, some of these records can be maintained in a format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The PI must assure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the PI must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

Arrowhead Pharmaceuticals, Inc. will inform the PI of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, or Arrowhead Pharmaceuticals, Inc. standards/procedures; otherwise, the retention period will default to 15 years.

The material to be stored shall include, but is not limited to, the following:

- Signed and dated copy of the final study protocol and any amendments
- Signed and dated letter of IRB/EC approval, letter of constitution of the IRB/EC and copies of any other correspondence relevant to the study with the IRB/EC or regulatory authorities
- The IRB/EC approved ICF
- Current *curriculum vitae* (signed and dated) of the PI and co-workers with major responsibilities in the trial
- Site Signature and Delegation of Responsibility Log
- Food and Drug Administration (FDA) Form 1572 (where applicable)
- Financial Disclosure Form(s)
- Blank case report form/eCRF
- Signed patient ICFs
- Laboratory reference ranges (signed and dated)
- The completed Clinical Trial Notification (CTN) Application Form (where applicable)
- The Final Study Report
- Clinical raw data including the Source Data Forms, all clinical laboratory report forms, patient eCRFs, drug accountability forms, and dispensing records, etc.

14. INFORMATION DISCLOSURE AND INVENTIONS

14.1. Ownership

[redacted]

14.2. Confidentiality

[redacted]

14.3. Publication

[redacted]

[redacted]

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APPENDIX 1: METABOLIC SYNDROME CRITERIA

Metabolic syndrome in this study will be defined as having at least 3 of the identified risk factors (Alberti et al., 2009).

Table 1. Criteria for Clinical Diagnosis of the Metabolic Syndrome

Measure	Categorical Cut Points
Elevated waist circumference*	Population- and country-specific definitions
Elevated triglycerides (drug treatment for elevated triglycerides is an alternate indicator†)	≥150 mg/dL (1.7 mmol/L)
Reduced HDL-C (drug treatment for reduced HDL-C is an alternate indicator†)	<40 mg/dL (1.0 mmol/L) in males; <50 mg/dL (1.3 mmol/L) in females
Elevated blood pressure (antihypertensive drug treatment in a patient with a history of hypertension is an alternate indicator)	Systolic ≥130 and/or diastolic ≥85 mm Hg
Elevated fasting glucose‡ (drug treatment of elevated glucose is an alternate indicator)	≥100 mg/dL

HDL-C indicates high-density lipoprotein cholesterol.

*It is recommended that the IDF cut points be used for non-Europeans and either the IDF or AHA/NHLBI cut points used for people of European origin until more data are available.

†The most commonly used drugs for elevated triglycerides and reduced HDL-C are fibrates and nicotinic acid. A patient taking 1 of these drugs can be presumed to have high triglycerides and low HDL-C. High-dose ω-3 fatty acids presumes high triglycerides.

‡Most patients with type 2 diabetes mellitus will have the metabolic syndrome by the proposed criteria.

APPENDIX 2: ELEVATED ALT OR WORSENING HEPATIC FUNCTION STUDY MODIFICATION AND PATIENT DISCONTINUATION RULES

Treatment-Emergent ALT	Treatment-Emergent Total Bilirubin (TBL)	Liver Symptoms	Action
Normal baseline: ALT >5× ULN Elevated baseline: ALT >3× baseline or >300 U/L (whichever occurs first)	Normal	None	Repeat ALT, AST, ALP, TBL, in 2–3 days Follow-up for symptoms.
Normal baseline: ALT >8× ULN Elevated baseline: ALT >5× baseline or >500 U/L (whichever occurs first)	Normal	None	Interrupt study drug. Initiate close observation and workup for competing etiologies. (see below) Study drug can be restarted only if an alternative etiology is identified and liver enzymes return to baseline.
Normal baseline: ALT >3× ULN Elevated baseline: ALT >2× baseline or >200 U/L (whichever occurs first)	TBL >2× ULN	None	Interrupt study drug. Initiate close observation and workup for competing etiologies. Study drug can be restarted only if an alternative etiology is identified and liver enzymes return to baseline.
Normal baseline: ALT >3× ULN Elevated baseline: ALT >2× baseline or >200 U/L (whichever occurs first)	Normal or elevated	Symptoms of clinical hepatitis - severe fatigue, nausea, vomiting, right upper quadrant pain	Interrupt study drug. Initiate close observation and workup for competing etiologies. Study drug should not be restarted

ALP=alkaline phosphatase; ALT= alanine aminotransferase; AST= aspartate aminotransferase; TBL=total bilirubin.
Source: Adapted from Chalasani, Naga and Regev, Arie et al. Drug-Induced Liver Injury in Subjects with Preexisting Chronic Liver Disease in Drug Development: How to Identify and Manage? Gastroenterology, Volume 151, Issue 6, 1046 – 1051

Close observation for potential drug-induced liver injury (DILI):

Within 72 hours, perform a complete history, physical, and liver biochemistries, including evaluation of:

- New or worsening signs and symptoms of clinical hepatitis such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia
- Concomitant medications, including acetaminophen, dietary supplements, herbal remedies, over-the-counter medications, recreational drug use, and special diets
- Alcohol consumption
- Exposure to environmental chemical agents
- Past medical history
- Complete review of systems
- Liver biochemistries including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin, direct bilirubin, and international normalized ratio (INR)
- Ruling out acute viral hepatitis type A, B C D and E; autoimmune or alcoholic hepatitis; nonalcoholic steatohepatitis (NASH); hypoxic/ischemic hepatopathy; and biliary tract disease (abdominal ultrasound).
- Consider gastroenterology or hepatology consultations

Evaluate patients 2 or 3 times a week for signs and symptoms of clinical hepatitis and obtain liver biochemistries until biochemistries stabilize or the trial drug has been discontinued and the patient is asymptomatic. If biochemistries stabilize and the patient is asymptomatic, monitor liver biochemistries once a week until they return to baseline. Analysis of such biochemistries may occur at local lab.

If the baseline liver enzymes are normal, follow the FDA guidance on “Drug- Induced Liver Injury: Premarketing Clinical Evaluation” for monitoring and managing patients if the patients experience elevations in liver enzymes during the clinical trial.

Patients who live far from study sites may be evaluated locally for history, physical exam, and laboratories, if the results are communicated promptly to the site Investigator.

Additional laboratory criteria to detect DILI:

- Interrupt the treatment and initiate DILI evaluation if:

  - Alkaline phosphatase (ALP)>2x ULN and direct bilirubin (DB) >2x ULN, if ALP and DB are normal at baseline
  - ALP >2x baseline and DB >2x baseline if they are elevated at baseline
  - ALP >2x baseline and DB >2x baseline and symptoms consistent with clinical hepatitis

- In the event of liver enzyme elevation, perform a DILI evaluation. If a clear etiology is established to account for the liver enzyme abnormalities, and the liver enzymes return back to baseline, the study drug may be restarted.
- If upon rechallenge, any magnitude of liver enzyme elevation recurs, the patient should cease the treatment drug and should be followed in the study. If a patient cannot be followed with laboratory or clinical monitoring, rechallenge should not be performed.

Stopping rule for progression to cirrhosis during the study:

APRI (using 40 U/L as ULN) will be calculated per SOA at baseline, Day 113 (Week 16), then every other dosing day (approximately every 168 days) or earlier if development of cirrhosis is suspected clinically, which may include development of varices, decompensating events or new thrombocytopenia and splenomegaly. Any increase from baseline to an APRI value >2.0 (must be confirmed on repeat) will trigger liver biopsy. For patients with baseline APRI >2.0 but who are eligible based on pre-dose biopsy, APRI will be calculated per SOA and a ≥50% increase from baseline (must be confirmed on repeat) will trigger liver biopsy.

Investigator may elect to confirm an elevated APRI as a biomarker of cirrhosis with MRE if available PRIOR to proceeding to biopsy. If MRE is NOT consistent with cirrhosis then biopsy is not indicated and the patient may stay on study. Alternatively, the Investigator may elect to proceed directly from APRI to biopsy.

If cirrhosis is detected on liver biopsy the patient will be discontinued from treatment and followed through EOS per SOA. This biopsy will represent EOS biopsy for purposes of endpoint determination. If cirrhosis is not confirmed on liver biopsy, patient may continue on treatment.

APPENDIX 3: PULMONARY FUNCTION MONITORING

Pulmonary function will be monitored closely throughout the AROAAT2001 study using spirometry, DLCO, periodic DSMB reviews, weekly Sponsor reviews and medical monitoring for pulmonary AEs.

If a patient in the AROAAT2001 study experiences an absolute decline of 10% or more in percent of predicted FEV_1 (must be confirmed on repeat within 30 days) from baseline with symptoms of chronic obstructive pulmonary disease (COPD) exacerbation,

OR experiences an absolute decline in percent of predicted FEV_1 by 10% (must be confirmed on repeat within 30 days) AND a decline in percent of predicted DLCO of at least 10%,

OR an absolute decline of percent of predicted FEV_1 of 20% or more (must be confirmed on repeat within 30 days), regardless of concurrent symptoms and regardless of any drop in percent of predicted DLCO,

they will be referred to a pulmonologist for possible initiation of augmentation therapy. Arrowhead will provide the therapy in any instance where the patient cannot access it through normal channels. Additionally, pulmonary monitoring visits for spirometry will be increased to approximately monthly.

If deemed necessary by the treating pulmonologist, AAT augmentation therapy may be initiated per locally approved package insert (typically weekly infusions at 60 mg/kg per dose) or equivalent for the duration of the AROAAT2001 study while the patient is on fazirsiran (or placebo) and for up to an additional 6 months after the patient's serum Z-AAT levels have returned to within 30% of their pre-dose Day 1 baseline serum AAT value OR until AAT levels have returned to within 30% of pre-dose Day 1 baseline and pulmonary function parameters (either DLCO or FEV_1) have stabilized (defined as 2 sequential measurements that are not declining) OR until cessation of augmentation therapy is deemed appropriate by treating pulmonologist. In the circumstance of patients in the study newly started on AAT augmentation therapy, measurement of serum AAT **may** increase in frequency from SOA to monthly or every 2 weeks. This measurement may be done with a Z-AAT specific assay or may require occasional AAT augmentation washout for measurement of endogenous production using the clinical AAT assay. In countries where augmentation is not available or not reimbursed, the Sponsor will work to make therapy available as part of the AROAAT2001 clinical study.

Each patient newly started on augmentation therapy during the study due to declines in FEV_1 and/or DLCO as described above will be reviewed by the DSMB to assess if the patient should continue to receive treatment with fazirsiran or placebo. Additionally, the DSMB will evaluate for imbalances in adverse changes in FEV_1, DLCO and pulmonary AEs in active versus placebo groups at each planned DSMB meeting.

APPENDIX 4: BIRTH CONTROL METHODS CONSIDERED HIGHLY EFFECTIVE

Methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods. Such methods include:

combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:

oral

intravaginal

transdermal

progestogen-only hormonal contraception associated with inhibition of ovulation:

oral

injectable

implantable

intrauterine device (IUD)

intrauterine hormone-releasing system (IUS)

bilateral tubal occlusion

vasectomized partner

sexual abstinence, defined as refraining from heterosexual intercourse only when this method is in alignment with the normal lifestyle of the patient

Birth control methods that result in a failure rate of more than 1% per year when used alone or in combination, thus are **NOT considered highly effective** include:

progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action

male or female condom with or without spermicide

cap, diaphragm, or sponge with spermicide

APPENDIX 5: DISCUSSION OF RISKS ASSOCIATED WITH STUDY ASSESSMENTS

Many of the assessments in the study i.e., liver biopsy, noninvasive hepatic imaging (FibroScan®), magnetic resonance imaging (MRI)/magnetic resonance elastography (MRE) and laboratory measurements are often part of a standard evaluation for liver disease severity made by a consulting physician treating a patient with AATD liver disease. Risk Assessments for patients are described in Section 4.8 of the study protocol. Assessments such as pulmonary function tests (PFTs), FibroScan® and MRE are noninvasive and involve minimal risk. Liver biopsy is an invasive assessment but is of critical importance in this study. AROAAT2001 is an innovative clinical study that requires liver biopsy to assess the novel histological primary endpoint and rating scale. Further assessment of the risk/benefit of the study assessments are summarized below:

- PFTs as used in the study (spirometry and DLCO) are standardly used to evaluate lung function in alpha-1 patients. A key benefit of this assessment is to track lung function during the study to evaluate for lung toxicity. There is very minimal risk associated with pulmonary function testing.

- FibroScan® and Magnetic Resonance Elastography (MRE) are noninvasive imaging modalities commonly used to evaluate liver disease in AATD and are of minimal risk for patients. The key clinical research benefit of these noninvasive imaging procedures is to track changes in liver stiffness related which in this study is being explored as markers for liver fibrosis and liver disease severity in patients with AATD-associated liver disease.

- Liver biopsy is the most invasive assessment in the study. Performance of liver biopsies is commonly part of the standard of care in patients with liver disease related to AATD as well as other adult liver diseases. While it is a common procedure, like any procedure it is associated with some risk. The risk of bleeding associated with biopsies requiring blood transfusion or hospitalization is 0.04% or less and the risk of less severe but clinically significant events (causing pain, tachycardia, or lower BP) is estimated at 0.2% (Rockey et al., 2009). Several larger studies show that complications and serious bleeding related to liver biopsy are overwhelmingly more likely in patients with serious clotting disorders, malignancy, and other serious health conditions that are described in the exclusion criteria for this study. The following risk mitigations are implemented in the protocol:

  – Liver biopsies are being performed by experienced physicians with appropriate local procedural credentials, in the setting of a hospital facility experienced with such procedures.

Measures of hemostatic function (e.g., platelets, international normalized ratio [INR]) will be conducted prior to biopsy, and may be used to exclude any patient at increased risk for procedural complications.

Thrombocytopenia and elevated INR are exclusionary for this study. Therefore, the risk of liver biopsy related complications in this study is acceptable and similar to other studies using liver histology as an endpoint.

Monitoring and recovery of the patient following the procedure will be consistent with locally accepted standards of clinical practice. Liver biopsy is the only method for obtaining the tissue specimens needed to evaluate the primary endpoint of the study.

Demonstration of an intrahepatic reduction of the disease-causing Z-AAT protein in response to fazirsiran is the pharmacologic and efficacy assessment which is most clinically relevant to patients with AATD-associated liver disease. Thus, the minimal risk of liver biopsy is justified in light of its use to obtain tissue samples required to assess clinically meaningful changes in intrahepatic Z-AAT protein and histology as represented by multiple key secondary study endpoints.

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