Cover Page for Protocol

Sponsor name:	Novo Nordisk A/S
NCT number	NCT04764448
Sponsor trial ID:	DCR-A1AT-201
Official title of study:	A Phase 2, Randomized, Double-blind, Placebo-controlled Study Investigating Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Two Dose Levels of Belcesiran in Patients with Alpha-1 Antitrypsin Deficiency-Associated Liver Disease
Document date:	02-May-2023

*Document date refers to the date on which the document was most recently updated.

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Protocol Title:	**A Phase 2, Randomized, Double-blind, Placebo-controlled Study Investigating Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Two Dose Levels of Belcesiran in Patients with Alpha-1 Antitrypsin Deficiency-Associated Liver Disease**
Protocol Number:	DCR-A1AT-201
Compound:	Belcesiran, also known as DCR-A1AT
Study Phase:	2
Short Title:	A Phase 2 Study of Belcesiran in Patients with AATLD
Study Name:	ESTRELLA
EudraCT Number:	2020-003313-35
NCT Number:	NCT04764448
Sponsor Name:	Dicerna Pharmaceuticals, Inc. 75 Hayden Ave Lexington, MA 02421 US (617) 621-8097
Version History:	Original Protocol (version 1.0) 03-Sep-2020 Amendment 1 (version 2.0) 04-Feb-2021 Amendment 2 (version 3.0) 24-May-2022 Amendment 3 (version 4.0) 02-May-2023

[redacted]

Redacted protocol
Includes redaction of personal identifiable information only.

Sponsor Signature Page

A Phase 2, Randomized, Double-blind, Placebo-controlled Study Investigating Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Two Dose Levels of Belcesiran in Patients with Alpha-1 Antitrypsin -Associated Liver Disease

Protocol Number: DCR-A1AT-201

Version: 4.0

Date: 02-May-2023

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Protocol Amendment 3, Summary of Changes

DOCUMENT HISTORY	
Document	**Date**
Amendment 3	02-May-2023
Amendment 2	24-May-2022
Amendment 1	04-Feb-2021
Original Protocol	03-Sep-2020

Amendment 3, 02-May-2023

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

Overall Rationale for the Amendment:

As the duration of this study will extend beyond January 2025, the protocol was updated to prepare for compliance with EU Clinical Trial Regulation 536/2014 (EU-CTR). The protocol has been harmonized across country-specific versions in the EU Member States, to the extent possible. Where not feasible, country-specific requirements are now located in Section 10.5. Additionally, the protocol has been updated to correct inconsistencies among the 3 study cohorts. Additional updates and clarifications were also included. A summary of all changes follows in the table below.

Description of Change	Brief Rationale	Affected Sections
Re-instate close monitoring rules for DILI	Close monitoring rules were initially added to address HPRA concerns. These rules will now apply across all countries.	7.1.1.2 Drug-Induced Liver Injury Monitoring
Study stopping rules were added	Study stopping rules were initially added to address HPRA concerns. These rules will now apply across all countries.	7.1.2 Study Stopping Rules
Stopping rules were updated and guidance on electrocardiogram findings are provided	To include electrocardiogram findings as judges by the investigator as a stopping rule for individual participants	7.1.1 Stopping Rules for Individual Participants 10.5 Electrocardiogram Findings
Added PFTs to the schedule of events	To harmonize schedule of activities with the US version of the protocol	1.3 Schedule of Activities

Made changes to the timing of collection of serum AFP, urinalysis, anti-drug antibodies, and blood biomarkers in Cohort 1 SoA	To harmonize schedule of activities between cohorts	1.3 Schedule of Activities
Made changes to the timing of collection of anti-drug antibodies, injection site inspection, hematology, chemistry, and coagulation, DLCO and spirometry, pregnancy test for WOCBP, weight, and serum and CRP in Cohort 2 SoA	To harmonize schedule of activities between cohorts	1.3 Schedule of Activities
Made changes to the timing of collection of urinalysis, anti-drug antibodies, SRGQ, and CLDQ, and added complement collection to Cohort 3 SoA	To harmonize schedule of activities between cohorts	1.3 Schedule of Activities
Specified the duration in which WOCBP must use contraception for at least 12 weeks after last dose of study drug	Was not clearly defined in previous versions	5.1 Inclusion Criteria 10.4 Contraceptive Guidance and Collection of Pregnancy Information
Clarified the definition of “pulmonary exacerbation”	To ensure consistency of reporting of exacerbations between sites	8.2.6 Other Pulmonary Information

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1. PROTOCOL SUMMARY

1.1. Synopsis

Protocol Title: **A Phase 2, Randomized, Double-blind, Placebo-controlled Study Investigating Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Two Dose Levels of Belcesiran in Patients with Alpha-1 Antitrypsin -Associated Liver Disease**

Short Title: **A Phase 2 Study of Belcesiran in Patients with AATLD**

Rationale:

Alpha-1 antitrypsin deficiency (AATD) is caused by inherited autosomal mutations in *SERPINA1*, the gene that encodes the alpha-1 antitrypsin (AAT) protein. The resultant mutant AAT protein (Z-AAT) is prone to misfolding and aggregation as homopolymers in hepatocytes. In individuals homozygous for the autosomal mutant AAT Z-allele (known as PiZZ patients), impaired degradation of the aggregated protein leads to putatively toxic accumulation of Z-AAT in the liver (toxic gain-of-function) and exerts continuous stress on the hepatocytes. Over time, this constant stress can lead to liver fibrosis, cirrhosis, and hepatocellular cancer. Currently, there is no treatment for this severe liver disease, aside from liver transplantation.

Dicerna Pharmaceuticals, Inc. is developing belcesiran, also known as DCR-A1AT, as a treatment for alpha-1 antitrypsin associated liver disease (AATLD) using an RNAi strategy to silence *SERPINA1*. This knockdown may lead to a reduction of total Z-AAT expression in the liver and, in turn, reduce the accumulation of toxic Z-AAT. Without the continuous putative toxic stimulus, the progression of AATLD in affected individuals may be halted, or potentially even reversed.

Belcesiran sodium (DCR-S1459, the drug substance for belcesiran), has been shown to significantly reduce AAT mRNA and AAT protein levels in transgenic mice and cynomolgus monkeys. Additionally, in the transgenic mouse model of AATLD, belcesiran sodium substantially reduced hepatic globules, indicating reduced accumulation of aggregated human Z-AAT protein.

The initial safety, tolerability, PK, and PD of belcesiran are being evaluated in an ongoing single-ascending-dose study in healthy volunteers (DCR-A1AT-101). To date, single doses of belcesiran have been well tolerated, with no SAEs or dose-limiting toxicities (DLT) reported. Study DCR-A1AT-201 is the first study of belcesiran in adults with AATLD. The overall goals of this study are to:

- demonstrate that belcesiran is safe and well-tolerated in adult PiZZ patients with liver fibrosis;
- investigate the effect of 2 dose levels of belcesiran on reduction of Z-AAT production in the liver, reflected by a reduction in circulating Z-AAT levels; and
- evaluate the effect of belcesiran on AATLD as assessed by liver biopsy and non-invasive markers of liver disease

Overall Design:

This study is a multiple dose, randomized, placebo-controlled, double-blind study to evaluate the safety, tolerability, PK, and PD of 2 belcesiran doses in adult patients with PiZZ AATLD (liver

fibrosis stage 1-4, METAVIR scoring system). Administration of the first dose of study intervention will occur on Day 1. Participants will return to the site for administration of study intervention and safety, tolerability, PK, and PD monitoring at specified time points as per the SoA through the End of Treatment (EOT) visit.

Intervention Groups and Duration:

The study will be conducted in 3 separate cohorts. Participants will be randomized in a 1:1 fashion to either Cohort 1 or 2. Once Cohort 1 and 2 have enrolled, enrollment of Cohort 3 will be initiated. Randomization will be stratified based on fibrosis stage (METAVIR Score F1, F2, F3, or F4) in all cohorts.

- Participants in Cohort 1 (N=8) will be randomized 3:1 to either belcesiran 210 mg or placebo and a liver biopsy will be performed at week 24 to assess the effect of belcesiran. In addition, participants will have the option to continue treatment for an additional 72 weeks so that the total treatment duration will be 96 weeks.
- Participants in Cohort 2 (N=8) will be randomized 3:1 to either belcesiran 210 mg or placebo and a liver biopsy will be performed at week 48 to assess the effect of belcesiran. In addition, participants will have the option to continue treatment for an additional 48 weeks so that the total treatment duration will be 96 weeks.
- Participants in Cohort 3 (N=30) will be randomized 2:1:2:1 to belcesiran 210 mg, the equivalent amount of placebo for belcesiran 210 mg, belcesiran 50 mg or the equivalent amount of placebo for belcesiran 50 mg. Participants will be blinded within each dose level. Participants will have a liver biopsy performed at week 24 to assess the effect of belcesiran and thereafter continue treatment until 96 weeks of treatment have been completed.

All participants in Cohorts 1 to 3 will have the option to undergo a liver biopsy at EOT/week 96. Biopsy findings will be correlated with changes in non-invasive markers of liver disease and liver stiffness.

In all cohorts, a follow up period of 48 weeks will follow EOT. In addition, Cohorts 1 and 2 will be followed for an additional 48 weeks to inform about the duration of potential treatment effects.

Study Population:

Key Inclusion Criteria:

1. Male or female age 18 to 75 years inclusive, at the time of signing the ICF.
2. Documented diagnosis of PiZZ-type AATD, confirmed by genotyping. Historical genotyping data may be used, if available.
3. AATLD, with a liver fibrosis score categorized as F1, F2, F3, or F4 in the METAVIR scoring system, documented by liver biopsy during Screening.
4. Post-bronchodilator $FEV_1 > 45\%$ of predicted at Screening. Participants receiving augmentation therapy on a regular basis and intending to continue augmentation therapy during the study are eligible to participate.
5. eGFR at Screening ≥ 60 mL/min normalized to 1.73 m^2 BSA.

6. Non-smokers (defined as having not smoked cigarettes daily for at least the preceding 12 months) with current non-smoking status confirmed by urine cotinine at Screening AND any previous smoking history prior to 12 months must be < 15 pack years, including use of e-cigarettes. Participants may be on nicotine replacement (patch or gum). A positive urine cotinine result due to nicotine replacement is acceptable for enrollment at the discretion of the Investigator.

Key Exclusion Criteria:

1. History of chronic liver disease other than non-alcoholic fatty liver disease from any cause other than PiZZ-type AATD.
2. Child-Pugh Score B or C or a previous hepatic decompensation event.
3. History of one single severe exacerbation of underlying lung disease in the year prior to randomization.

   A severe exacerbation is defined as an exacerbation that requires hospitalization or a visit to the emergency room.

4. History of rapid decline in pulmonary function, as assessed by the Investigator.

Disclosure Statement:

This is a parallel-cohort, placebo-controlled, treatment study investigating belcesiran in patients with AATLD. Participants and Investigators will be blinded to treatment. Complete details, including any Sponsor team members who are planned to be unblinded, will be presented in the Study Specific Blinding Plan.

Number of Participants:

Up to a total number of 16 participants will be enrolled in Cohorts 1 and 2. A total of 30 participants will be enrolled in Cohort 3.

Data Safety Monitoring Committee:

A DSMC will be convened to review safety data at predefined intervals, including at the time of a planned interim analysis, to ensure the safety of the participants in all cohorts. The timing and scope of the review meetings will be defined in the DSMC charter.

Objectives and Endpoints:

Objectives	Endpoints
Primary (Cohorts 1 and 2)	
1. To evaluate the safety and tolerability of multiple doses of belcesiran in patients with AATLD	1. The incidence and nature of TEAEs, and the change from Baseline in PFTs, 12 -lead ECGs, physical examination findings, vital signs, and clinical laboratory tests
2. To characterize the PD of belcesiran in patients with AATLD	2. Changes from baseline to weeks 24 (Cohort 1)/48 (Cohort 2) in serum AAT protein concentrations
Primary (Cohort 3)	
1. To characterize the PD of belcesiran in patients with AATLD	1. Change from baseline to week 24 in serum Z-AAT protein levels 2. Change from baseline to week 24 in liver Z-AAT protein levels
Secondary	
1. To characterize the PK of belcesiran in the plasma of patients with AATLD	1. PK profile of belcesiran
2. To assess the effect of belcesiran on liver histology in patients with AATLD	2. Change from Baseline up until week 96 in liver fibrosis 3. Change from Baseline up until week 96 in diastase-resistant PAS-positive AAT globules
Exploratory	
1. To assess the effect of belcesiran on liver stiffness in patients with AATLD	1. Change from Baseline up until week 96 in FibroScan® score
2. To assess the effect of belcesiran on liver fibrosis and/or inflammation in patients with AATLD	2. Change from Baseline up until week 96 in ELF score 3. Change from Baseline up until week 96 in CK-18

1.2. Schema

Figure 11 Dose Regimens by Cohort

Cohort 1

	Visit #	Dose #	Day	Week
Treatment Period	1	1	1	
	2		2	
	3		3	
	4		15	
	5	2	29	
	6		30	
	7	3	57	
	8	4	85	
	9	5	113	
	10	6	141	
	11	7	169	**24**
Optional Treatment Period	12		211*	
	13	8	253	
	14		295*	
	15	9	337	**48**
	16	10	421	
	17	11	505	**72**
	18	12	589	
	19	13	673	**96**
Follow-up Period	18		757	
	19		841	**120**
	20		925	
	21		1009	**144**
	22		1177	
	23		1345	**192**

Required Dosing Visits in yellow
Optional Dosing Visits in blue
Follow-up visits in gray
* Phone visit

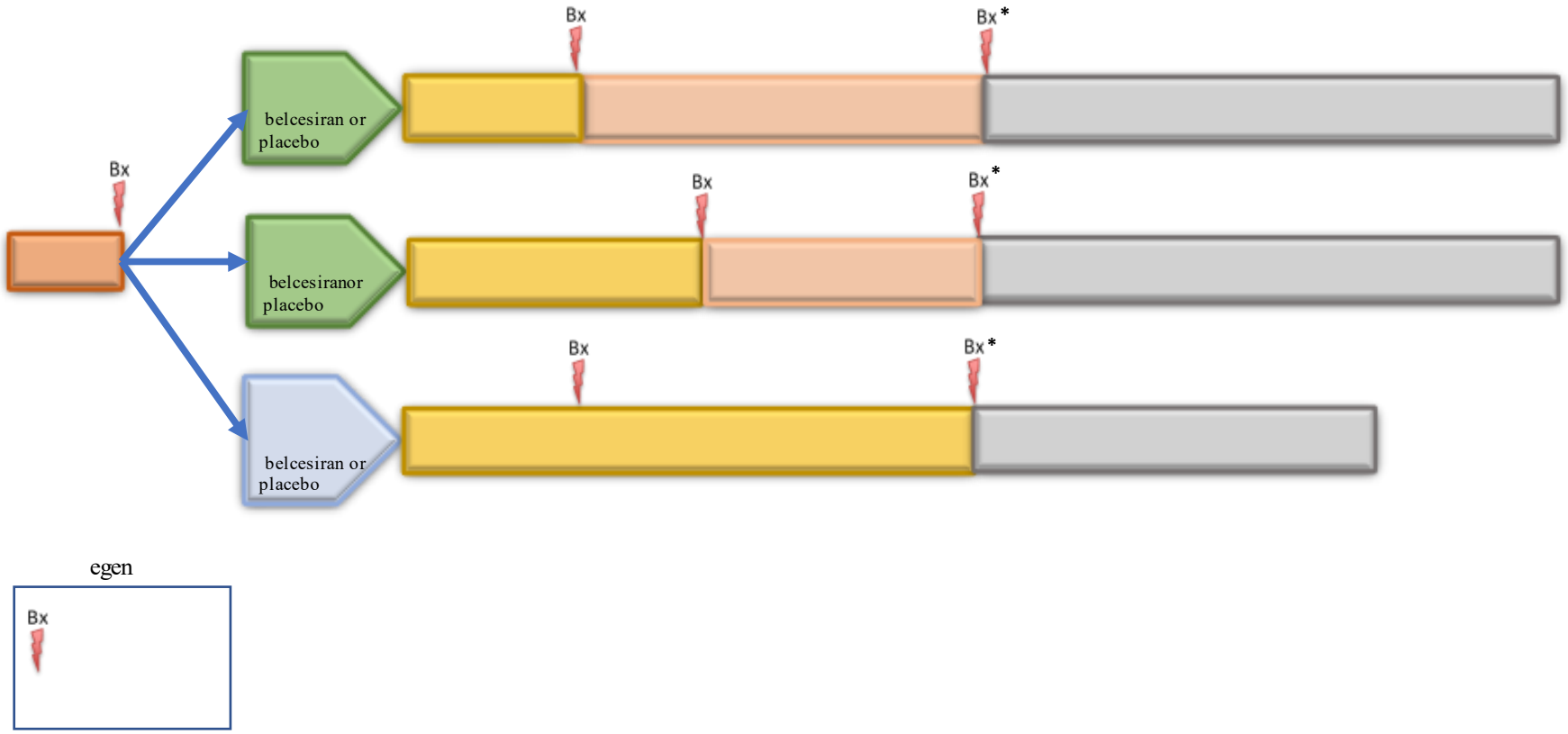
Cohort 2

	Visit #	Dose #	Day	Week
Treatment Period	1	1	1	
	2		2	
	3		3	
	4		15	
	5	2	29	
	6		30	
	7	3	57	
	8	4	85	
	9	5	113	
	10	6	141	
	11	7	169	**24**
	12	8	197	
	13	9	225	
	14	10	253	
	15	11	281	
	16	12	309	
	17	13	337	**48**
Optional Treatment Period	18	14	421	
	19	15	505	**72**
	20	16	589	
	21	17	673	**96**
Follow-up Period	22		757	
	23		841	**120**
	24		925	
	25		1009	**144**
	26		1177	
	27		1345	**192**

Cohort 3

	Visit #	Dose #	Day	Week
Treatment Period	1	1	1	
	2		2	
	3		3*	
	4		15*	
	5	2	29	
	6		30*	
	7	3	57	
	8	4	85	
	9	5	113	
	10	6	141	
	11	7	169	**24**
	12		211*	
	13	8	253	
	14		295*	
	15	9	337	**48**
	16	10	421	
	17	11	505	
	18	12	589	
	19	13	673	**96**
Follow-up Period	20		757	
	21		841	**120**
	22		925	
	23		1009	**144**

Figure 22: Study Schematic



1.3. Schedules of Activities

Table 11: Screening Period Schedule of Activities

Study Week [a]	−6	−4
Visit Window	±2 weeks	±1 week
Procedure/Assessment		
Informed consent [b]	X	
Assess I/E criteria	X	
PiZZ genotyping [c]	X	
Demographics	X	
General medical history	X	
Medication history	X	
Smoking history	X	
Pulmonary X-ray [d]	X	
Vital signs	X	
Complete physical examination [e]	X	
Height and Weight	X	
12-lead ECG	X	
Screening labs [f]	X	
Hematology/ chemistry/ coagulation [g]	X	
Serum AFP	X	
Blood biomarkers of fibrosis [h]	X	
Blood biomarkers of inflammation	X	
Samples for future analysis [i]	X	
Serum pregnancy test for WOCBP	X	
Urinalysis	X	
Urine cotinine	X	
FibroScan® [j]	X	
MRE [j]	X	
DLCO & Spirometry [k, l]	X	
Serum AAT & CRP	X	
Liver biopsy [m]		X
Record AEs & SAEs	X	X

Abbreviations: AAT: alpha-1 antitrypsin; AE: adverse event; AFP: alpha fetoprotein; CRP: C-reactive protein; DLCO: diffusion capacity of the lungs for carbon monoxide; ECG: electrocardiogram; FSH: follicle stimulating hormone; I/E: inclusion/exclusion; MRE: magnetic resonance elastography; PiZZ: autosomal homozygous mutant AAT Z allele genotype; SAE: serious adverse event; WOCBP: women of childbearing potential.

a The screening period may take up to 6 weeks (±2 weeks) but can be condensed into a shorter time period if possible.
b Potential participants will provide informed consent prior to the initiation of any study-related screening assessment. The signing of the ICF will initiate the screening window.
c If a participant has been previously genotyped, historical data may be used if documentation is available.
d If the participant has undergone x-ray imaging in a stable condition within the prior 4 weeks, that x-ray may be used if the images are available.
e A complete physical examination is defined in Section 8.2.2.
f To include viral serology, urine drug screen, HbA1c, FSH (as needed to confirm post-menopausal status), antimitochondrial antibodies (if primary biliary cholangitis is suspected) and testing to rule out autoimmune hepatitis (may include total IgG or gamma-globulin levels and/or serologic markers), and cotinine as needed. If a participant has documented screening for the hepatitis B virus, hepatitis C virus, and human immunodeficiency virus in the past 3 months, historical data may be used. See Section 10.2 for details.
g See Section 10.2 for a detailed list of clinical laboratory parameters.
h To include biochemical fibrosis index, APRI, Fib-4, Enhanced Liver Fibrosis Score, and other serum biomarkers of liver fibrosis (see Section 8.1.3).
i Samples for future analysis will be collected at selected visits only if a participant has consented to future analysis.
j Participants must be fasting for a minimum of 3 hours prior to the FibroScan and 6 hours prior to the MRE (Cohorts 1 & 2 only) being performed. Please ensure fasting begins at the site after the participant has signed the ICF. If the clinical site does not have access to the required equipment or technology, MRE assessments would be optional.
k Spirometry (FEV_1 and FVC) will be performed pre-bronchodilator and post-bronchodilator. The order of testing for recorded values should be 1) administer bronchodilator, 2) immediately perform DLCO, 3) perform spirometry.
l When screening pulmonary function tests are being performed, historical information including the number and severity of pulmonary exacerbations, resting peripheral oxygen saturation, smoking status including number of cigarettes per day, and use of AAT replacement therapy should be collected from the participant. The Modified Medical Research Council dyspnea scale should also be assessed.
m Liver biopsy to be performed only after all other screening assessments are completed.

Table 22: Treatment Period Schedule of Activities – Cohort 1

Dose #	1				2		3	4	5	6	EOT/ 7		8 [a]		9 [a]	10 [a]	11 [a]	12 [a]	13/ ET [a1,b]
Study day	1	2	3	15	29	30 [c]	57	85	113	141	169	211	253	295	337	421	505	589	673
Visit	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	P12[d]	V13	P14[d]	V15	V16	V17	V18	V19
Visit window (days)	–	–	–	±2	±2	–	±5	±5	±5	±5	±5	±5	±7	±5	±7	±7	±7	±7	±7
Confirm I/E criteria	X																		
Randomization	X																		
Study intervention	X[e]				X		X	X	X	X	X		X		X	X	X	X	X
Injection Site Inspection	X				X		X	X	X	X	X		X		X	X	X	X	X
DLCO & Spirometry [f]	X				X		X	X	X	X	X		X		X	X	X	X	X
Pulmonary Information [g]	X				X		X	X	X	X	X		X		X	X	X	X	X
Hematology/chemistry/ coagulation [h]	X	X	X	X	X	X	X	X	X	X	X		X		X	X	X	X	X
Pregnancy test for WOCBP [i]	X				X		X	X	X	X	X		X		X	X	X	X	X
Complement [j]	X	X									X								X
Serum AFP [k]	X				X			X			X		X		X	X	X	X	X
Urinalysis [l]	X			X	X			X			X		X		X				X
UACR & UPCR [l]	X			X	X			X			X		X		X				X
Serum AAT & CRP [m]	X			X	X		X	X	X	X	X		X		X	X	X	X	X
Antidrug antibodies [n]	X				X			X			X		X		X				X
Blood biomarkers of fibrosis [o]	X				X			X			X				X		X		X
Blood biomarkers of inflammation	X				X			X			X				X		X		X
Samples for future analysis [p]	X										X				X				X

Dose #	**1**				**2**		**3**	**4**	**5**	**6**	**EOT/ 7**		**8** [a]		**9** [a]	**10** [a]	**11** [a]	**12** [a]	**13/ ET** [a1,b]
Study day	**1**	**2**	**3**	**15**	**29**	**30** [c]	**57**	**85**	**113**	**141**	**169**	**211**	**253**	**295**	**337**	**421**	**505**	**589**	**673**
Visit	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**P12**[d]	**V13**	**P14**[d]	**V15**	**V16**	**V17**	**V18**	**V19**
Visit window (days)	–	–	–	±2	±2	–	±5	±5	±5	±5	±5	±5	±7	±5	±7	±7	±7	±7	±7
Plasma PK [q]	X	X	X	X	X	X	X	X	X	X	X		X		X	X	X	X	X
Vital signs [r]	X	X	X	X	X	X	X	X	X	X	X		X		X	X	X	X	X
Physical examination	X	X[s]	X[s]	X[s]	X[s]	X[s]	X	X[s]	X[s]	X[s]	X		X[s]		X[s]	X[s]	X[s]	X[s]	X
Weight	X										X				X				X
12-lead ECG [t]	X	X		X	X	X	X	X	X	X	X				X		X		X
CLDQ [u]	X							X			X				X				X
SGRQ [u]	X							X			X				X				X
FibroScan [v]											X				X				X
MRE [v]											X				X				X
Liver biopsy											X								X [w]
Record AEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Record SAEs [x]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications/procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Attend visit fasting [y]	X										X				X				X

Abbreviations: AAT: alpha-1 antitrypsin; AE: adverse event; AFP: alpha fetoprotein; BMI: body mass index; CLDQ: Chronic Liver Disease Questionnaire; COVID-19: corona virus disease 2019; CRP: C-reactive protein; DLCO: diffusion capacity of the lungs for carbon monoxide; DSMC: Data Safety Monitoring Committee; ECG: electrocardiogram; EOT: end of treatment; ET: early termination; FEV1: forced expiratory volume in one second; F/U: follow-up; I/E: Inclusion/Exclusion; FVC: forced vital capacity; MRE: magnetic resonance elastography; PK: pharmacokinetics; SAE: serious adverse event; SGRQ: St. George's Respiratory Questionnaire; UACR: urine albumin-creatinine ratio; UPCR: urine protein-creatinine ratio; WOCBP: women of childbearing potential.

a Visits indicated are optional dosing visits that can be performed if the participant consents. If the participant does not want to continue with the optional dosing, they will receive their final dose at EOT/D169 and then continue into follow up.

b Participants who terminate the stu y prematurely early termination "ET" will un ergo all activities as escribe un er D673 with the exception of dosing.

c Day 30 will always be the day after Day 29, regardless of when Day 29 occurs within the ± 2-day visit window

d Any visit marke with a "P" in the "Visit" row can be complete as a phone visit. The Investigator can choose to have site visits on these days if deemed necessary.
e The first dose of study intervention must be administered at the clinical site.
f Post-bronchodilator values for DLCO and spirometry (FEV_1 and FVC) will be recorded. The order of testing for recorded values should be 1) administer bronchodilator, 2) immediately perform DLCO, and 3) perform spirometry. On dosing days, DLCO and spirometry will be performed predose.
g Collect and record the number and severity of pulmonary exacerbations since the last PFT assessment, resting peripheral oxygen saturation, smoking status including number of cigarettes per day, and use of AAT replacement therapy since the last visit. Assess and record the Modified Medical Research Council dyspnea scale.
h For Dose 1 only, blood samples for hematology, clinical chemistry, and coagulation studies will be collected predose and at 6 hours (± 30 minutes) postdose. At other dosing visits, these samples will only be collected predose. See Section 10.2 for a detailed list of clinical laboratory parameters.
i A urine pregnancy test will be performed prior to dosing. If the result is positive, a confirmatory serum pregnancy test will be performed. Dosing should not occur unless a negative result is obtained with the confirmatory serum pregnancy test in consultation with the Sponsor.
j Samples for complement analysis will be collected surrounding Dose 1 only. For Dose 1, samples will be collected predose, at 2 and 6 hours (±30 minutes), and 24 hours (± 1 hour) postdose. At EOT, samples will be collected at the same time as all other sample collections.
k The AFP sample should be collected predose.
l Urine sample will be collected predose.
m The serum AAT and CRP samples should be collected predose. Blood samples for determination of CRP will be collected at the same time as those for determination of serum AAT.
n At all visits, the blood sample for antidrug antibodies will be collected predose.
o Blood samples for biomarkers of fibrosis will be collected predose on dosing days.
p Samples for future analysis will be collected at selected visits only if a participant has consented to future analysis.
q Plasma samples for PK analysis will be collected predose and at 5, 15, 30, and 60 minutes and 2, 4, 6, 8, 12, and 24 hours postdose for Doses 1 and 2 only. Single samples will be collected predose for other doses and at other visits as indicated. The following windows are allowed:
    - **Predose**
    - **5 and 15 minutes postdose: ± 3 minutes**
    - **2, 4, 6, and 8 hours postdose: ± 15 minutes**
    - **30 and 60 minutes postdose: ± 5 minutes**
    - **12 and 24 hours postdose: ± 1 hour**
r Vital sign measurements will include blood pressure, pulse rate, respiratory rate, and temperature (collected per local standard of care). For Dose 1 and 2, vital sign measurements will be performed predose, at the end of injection, at 30 (± 10) and 60 (± 15) minutes, at 2 (± 30 minutes), 6 (± 30 minutes), and 24 hours (± 1 hour) postdose. At other dosing visits, vital signs will be measured predose. If multiple assessments are due at the same time, the order of priority should be ECG, vitals, PK, and then other assessments; with PK sampling being performed at the nominal time point.
s A focused physical examination will be performed (see Section 8.2.2) for Dose 1 and 2 only, ECG will be performed predose and at 30 minutes and 6 hours postdose. For other doses, a single ECG will be performed predose. If multiple assessments are due at the same time, the order of priority should be ECG, vitals, PK, and then other assessments, with PK sampling being performed at the nominal time point.
t At Dose 1, the required questionnaires will be performed predose. See Section 8.1.6 and 8.1.7.
u The EOT FibroScan and MRE will be performed prior to the EOT liver biopsy. Participants must be fasting for a minimum of 3 hours prior to the FibroScan and 6 hours prior to the MRE being performed. If Screening MRE is done, it is expected that an MRE is done at EOT. If a clinical site does not have access to the required equipment or technology, MRE assessments would be optional.
v At ET, a liver biopsy will be optional.
w Serious AEs and treatment-emergent SAEs will be assessed until 30 days after the last day of study participation. Ongoing SAEs will be followed until resolution or until resolved with sequalae.
x Participants should be fasting for a minimum of 8 hours prior to selected visits.

Table 33: Treatment Period Schedule of Activities – Cohort 2

Dose No.	**1**				**2**		**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**EOT/ 13**	**14[a]**	**15[a]**	**16[a]**	**17/ ET [a1,b]**
Study Day	**1**	**2**	**3**	**15**	**29**	**30 [c]**	**57**	**85**	**113**	**141**	**169**	**197**	**225**	**253**	**281**	**309**	**337**	**421**	**505**	**589**	**673**
Visit window (days)	–	–	–	**±2**	**±2**	–	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±7**	**±7**	**±7**	**±7**
Visit	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21
Confirm I/E criteria	X																				
Randomization	X																				
Study intervention	X[d]				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Injection Site Inspection	X				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
DLCO & Spirometry [e]	X				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pulmonary Information [f]	X				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology/chemistry/ coagulation [g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test for WOCBP [h]	X				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Complement [i]	X	X															X				X
Serum AFP [j]	X				X			X			X			X			X	X	X	X	X
Urinalysis [k]	X			X	X			X			X			X			X				X
UACR & UPCR [k]	X			X	X			X			X			X			X				X
Serum AAT & CRP [l]	X			X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Antidrug antibodies [m]	X				X			X			X			X			X				X
Blood biomarkers of fibrosis [n]	X				X			X			X						X		X		X
Blood biomarkers of inflammation	X				X			X			X						X		X		X

Dose No.	**1**				**2**		**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**EOT/ 13**	**14[a]**	**15[a]**	**16[a]**	**17/ ET [a1,b]**
Study Day	**1**	**2**	**3**	**15**	**29**	**30 [c]**	**57**	**85**	**113**	**141**	**169**	**197**	**225**	**253**	**281**	**309**	**337**	**421**	**505**	**589**	**673**
Visit window (days)	–	–	–	**±2**	**±2**	–	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±5**	**±7**	**±7**	**±7**	**±7**
Visit	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21
Samples for future analysis [o]	X										X						X				X
Plasma PK [p]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs [q]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X[r]	X[r]	X[r]	X[r]	X[r]	X[r]	X	X[r]	X[r]	X	X[r]	X[r]	X[r]	X[r]	X[r]	X	X[r]	X[r]	X[r]	X
Weight	X										X						X				X
12-lead ECG [s]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X
CLDQ [t]	X							X			X			X			X				X
SGRQ [t]	X							X			X			X			X				X
FibroScan® [u]											X						X				X
MRE [u]											X						X				X
Liver biopsy																	X				X [v]
Record AEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Record SAEs [w]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications/ procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Attend visit fasting [x]	X										X						X				X

Abbreviations: AAT: alpha-1 antitrypsin; AE: adverse event; AFP: alpha fetoprotein; F/U: follow-up; CLDQ: Quality of Life Index for Patients with Chronic Liver Disease; CRP: C-reactive protein; DLCO: diffusion capacity of the lungs for carbon monoxide; DSMC: Data Safety Monitoring Committee; ECG: electrocardiogram; EOT: end of treatment; ET: early termination; I/E: Inclusion/Exclusion; MRE: magnetic resonance elastography; PE: physical examination; PK: pharmacokinetics; SAE: serious adverse event; SGRQ: St. George's Respiratory Questionnaire; UACR: urine albumin-creatinine ratio; UPCR: urine protein-creatinine ratio; WOCBP: women of childbearing potential; Z-AAT: the mutant AAT protein.

a Visit indicated are optional dosing visits that can be performed if the participant consents. If the participant does not want to continue with the optional dosing, they will receive their final dose at EOT/ D337 and then continue into follow up.

b Participants who terminate the stu y prematurely early termination "ET" will un ergo all activities as described under D673, with the exception of dosing.

c Day 30 will always be the day after Day 29, regardless of when Day 29 occurs within the ± 2-day visit window.
d The first dose of study intervention must be administered at the clinical site.
e Post-bronchodilator values for DLCO and spirometry (FEV_1 and FVC) will be recorded. The order of testing for recorded values should be 1) administer bronchodilator, 2) immediately perform DLCO, and 3) perform spirometry. On dosing days, DLCO and spirometry will be performed predose.
f Collect and record the number and severity of pulmonary exacerbations since the last PFT assessment, resting peripheral oxygen saturation, smoking status including number of cigarettes per day, and use of AAT replacement therapy since the last visit. Assess and record the Modified Medical Research Council dyspnea scale.
g For Dose 1 only, blood samples for hematology, clinical chemistry, and coagulation studies will be collected predose and at 6 hours (± 30 minutes) postdose. At other dosing visits, these samples will be collected predose only. See Section 10.2 for a detailed list of clinical laboratory parameters.
h A urine pregnancy test will be performed. If the result is positive, a confirmatory serum pregnancy test will be performed. Dosing should not occur unless a negative result is obtained with the confirmatory serum pregnancy test in consultation with the Sponsor.
i Samples for complement analysis will be collected surrounding Dose 1 only. For Dose 1, samples will be collected predose, at 2 and 6 hours (± 30 minutes), and 24 hours (± 1 hour) postdose. At EOT, samples will be collected at the same time as all other sample collections.
j The AFP sample should be collected predose.
k Urine sample will be collected predose.
l The serum AAT and CRP sample should be collected predose. Blood samples for determination of CRP will be collected at the same time as those for determination of serum AAT.
m At all visits, the blood sample for antidrug antibodies will be collected predose.
n Blood samples for biomarkers or fibrosis will be collected predose on dosing days.
o Samples for future analysis will be collected at selected visits only if a participant has consented to future analysis.
p Plasma samples for PK analysis will be collected predose and at 5, 15, 30, and 60 minutes and 2, 4, 6, 8, 12, and 24 hours postdose for Doses 1 and 2 only. Single samples will be collected predose for other doses and at other visits as indicated. The following windows are allowed:
    - **Predose**
    - **5 and 15minutes postdose: ± 3 minutes**
    - **2, 4, 6, and 8 postdose: ± 15 minutes**
    - **30 and 60 minutes postdose: ± 5 minutes**
    - **12 and 24 hours postdose: ± 1 hour**
q Vital sign measurements will include blood pressure, pulse rate, respiratory rate, and temperature (collected per local standard of care). For Dose 1 and 2, vital sign measurements will be performed predose, at the end of injection, at 30 (± 10) and 60 (± 15) minutes, at 2 (± 30 minutes), 6 (± 30 minutes), and 24 hours (± 1 hour) postdose for Dose 1 only. At other dosing visits, vital signs will be measured predose. If multiple assessments are due at the same time, the order of priority should be ECG, vitals, PK, and then other assessments; with PK sampling being performed at the nominal time point.
r Focused physical examination (see section 8.2.2).
s For Dose 1 and 2 only, ECG will be performed predose and at 30 minutes and 6 hours postdose. For other doses, a single ECG will be performed predose. If multiple assessments are due at the same time, the order of priority should be ECG, vitals, PK, and then other assessments, with PK sampling being performed at the nominal time point.
t At Dose 1, the required questionnaires will be performed predose.
u The EOT FibroScan and MRE will be performed prior to the EOT liver biopsy. Participants must be fasting for a minimum of 3 hours prior to the FibroScan and 6 hours prior to the MRE being performed. If Screening MRE is done, it is expected that an MRE is done at EOT. If a clinical site does not have access to the required equipment or technology, MRE assessments would be optional.
v Liver biopsy is optional at V21.
w Serious AEs and treatment-emergent SAEs will be assessed until 30 days after the last day of study participation, ongoing SAEs will be followed until resolution or until resolved with sequalae.
x Participants should be fasting for a minimum of 8 hours prior to selected visits.

Table 44: Treatment Period Schedule of Activities – Cohort 3

Dose No.	**1**				**2**		**3**	**4**	**5**	**6**	**7**		**8**		**9**	**10**	**11**	**12**	**EOT/ ET [al] 13**
Study Day	**1**	**2**	**3**	**15**	**29**	**30 [b]**	**57**	**85**	**113**	**141**	**169**	**211**	**253**	**295**	**337**	**421**	**505**	**589**	**673**
Visit	**V1**	**V2**	**P3[c]**	**P4[c]**	**V5**	**P6[c]**	**V7**	**V8**	**V9**	**V10**	**V11**	**P12[c]**	**V13**	**P14[c]**	**V15**	**V16**	**V17**	**V18**	**V19**
Visit window (days)	**–**	**–**	**–**	**± 3**	**± 5**	**–**	**± 5**	**± 5**	**± 5**	**± 5**	**± 5**	**± 5**	**± 7**	**± 5**	**± 7**	**± 7**	**± 7**	**± 7**	**± 7**
Confirm I/E criteria	X																		
Randomization	X																		
Study intervention	X[d]				X		X	X	X	X	X		X		X	X	X	X	X
Injection Site Inspection	X				X		X	X	X	X	X		X		X	X	X	X	X
DLCO & Spirometry [e]	X				X		X	X	X	X	X		X		X	X	X	X	X
Pulmonary Information [f]	X				X		X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology/chemistry/ coagulation [g]	X	X			X		X	X	X	X	X		X		X	X	X	X	X
Pregnancy test for WOCBP [h]	X				X		X	X	X	X	X		X		X	X	X	X	X
Complement [i]	X	X									X				X				X
Serum AFP [j]	X							X			X		X		X	X	X	X	X
Urinalysis [k]	X				X			X			X		X		X				X
UACR & UPCR [k]	X				X			X			X		X		X				X
Serum AAT & CRP [l]	X				X		X	X	X	X	X		X		X	X	X	X	X
Antidrug antibodies [m]	X				X			X			X		X		X				X
Blood biomarkers of fibrosis [n]	X				X			X			X				X		X		X
Blood biomarkers of inflammation	X				X			X			X				X		X		X
Samples for future analysis [o]	X										X				X				X
Plasma PK [p]	X	X			X		X	X	X	X	X		X		X	X	X	X	X
Vital signs [q]	X	X			X		X	X	X	X	X		X		X	X	X	X	X

Dose No.	**1**				**2**		**3**	**4**	**5**	**6**	**7**		**8**		**9**	**10**	**11**	**12**	**EOT/ ET** [a] **13**
Study Day	**1**	**2**	**3**	**15**	**29**	**30** [b]	**57**	**85**	**113**	**141**	**169**	**211**	**253**	**295**	**337**	**421**	**505**	**589**	**673**
Visit	**V1**	**V2**	**P3**[c]	**P4**[c]	**V5**	**P6**[c]	**V7**	**V8**	**V9**	**V10**	**V11**	**P12**[c]	**V13**	**P14**[c]	**V15**	**V16**	**V17**	**V18**	**V19**
Visit window (days)	–	–	–	**± 3**	**± 5**	–	**± 5**	**± 5**	**± 5**	**± 5**	**± 5**	**± 5**	**± 7**	**± 5**	**± 7**	**± 7**	**± 7**	**± 7**	**± 7**
Physical examination	X	X [r]			X[r]		X[r]	X	X[r]	X[r]	X		X [r]		X	X[r]	X [r]	X[r]	X
Weight	X										X				X				X
12-lead ECG [s]	X	X			X			X			X		X		X		X		X
CLDQ [t]	X							X			X		X		X				X
SGRQ [t]	X							X			X		X		X				X
FibroScan® [u]											X				X				X
Liver biopsy											X								X[v]
Record AEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Record SAEs [w]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications/ procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Attend visit fasting [x]	X										X				X				X

Abbreviations: AAT: alpha-1 antitrypsin; AE: adverse event; AFP: alpha fetoprotein; F/U: follow-up; CLDQ: Quality of Life Index for Patients with Chronic Liver Disease; CRP: C-reactive protein; DLCO: diffusion capacity of the lungs for carbon monoxide; DSMC: Data Safety Monitoring Committee; ECG: electrocardiogram; EOT: end of treatment; ET: early termination; I/E: Inclusion/Exclusion; PE: physical examination; PK: pharmacokinetics; SAE: serious adverse event; SGRQ: St. George's Respiratory Questionnaire; UACR: urine albumin-creatinine ratio; UPCR: urine protein-creatinine ratio; WOCBP: women of childbearing potential; Z-AAT: the mutant AAT protein.

a Participants who terminate the stu y prematurely early termination "ET" will un ergo all activities as escribe un er EOT (Note: ± 14-day window does not apply to ET).
b Day 30 will always be the day after Day 29, regardless of when Day 29 occurs within the ± 2-day visit window.
c Any visit marke with a "P" in the "Visit" row can be completed as a phone visit. Investigator can choose to have site visits on these days if deemed necessary.
d The first dose of study intervention must be administered at the clinical site.
e Post-bronchodilator values for DLCO and spirometry (FEV_1 and FVC) will be recorded. The order of testing for recorded values should be 1) administer bronchodilator, 2) immediately perform DLCO, and 3) perform spirometry. On dosing days, DLCO and spirometry will be performed predose.
f Collect and record the number and severity of pulmonary exacerbations since the last PFT assessment, resting peripheral oxygen saturation, smoking status including number of cigarettes per day, and use of AAT replacement therapy since the last visit. Assess and record the Modified Medical Research Council dyspnea scale.

g For Dose 1 only, blood samples for hematology, clinical chemistry, and coagulation studies will be collected predose and at 6 hours (± 30 minutes) postdose. At other dosing visits, these samples will be collected predose. See Section 10.2 for a detailed list of clinical laboratory parameters.

h A urine pregnancy test will be performed. If the result is positive, a confirmatory serum pregnancy test will be performed. Dosing should not occur unless a negative result is obtained with the confirmatory serum pregnancy test in consultation with the Sponsor.

i Samples for complement analysis will be collected surrounding Dose 1 only. For Dose 1, samples will be collected predose, at 2 and 6 hours (± 30 minutes), and 24 hours (± 1 hour) postdose. At D169, D337, and EOT, samples will be collected at the same time as all other sample collections.

j The AFP sample should be collected predose.

k Urine sample will be collected predose.

l The serum AAT and CRP sample should be collected predose. Blood samples for determination of CRP will be collected at the same time as those for determination of serum AAT.

m At all visits, the blood sample for antidrug antibodies will be collected predose.

n Blood samples for biomarkers or fibrosis will be collected predose on dosing days.

o Samples for future analysis will be collected at selected visits only if a participant has consented to future analysis.

p Plasma samples for PK analysis will be collected predose and at 5, 15, 30, and 60 minutes and 2, 4, 6, 8, and 12 hours postdose for Doses 1 and 2 only; and at 24 hours for postdose for Dose 1 only. Single samples will be collected predose for other doses and at other visits as indicated. The following windows are allowed:

- **Predose**
- **5 and 15minutes postdose: ± 3 minutes**
- **2, 4, 6, and 8 postdose: ± 15 minutes**
- **30 and 60 minutes postdose: ± 5 minutes**
- **12 and 24 hours postdose: ± 1 hour**

q Vital sign measurements will include blood pressure, pulse rate, respiratory rate, and temperature (collected per local standard of care). For Dose 1 and 2, vital sign measurements will be performed predose, at the end of injection, at 30 (± 10) and 60 (± 15) minutes, at 2 (± 30 minutes), 6 (± 30 minutes), and 24 hours (± 1 hour) postdose for Dose 1 only. At other dosing visits, vital signs will be measured predose. If multiple assessments are due at the same time, the order of priority should be ECG, vitals, PK, and then other assessments; with PK sampling being performed at the nominal time point.

r Focused physical examination (see Section 8.2.2)

s For Dose 1 and 2 only, ECG will be performed predose and at 30 minutes and 6 hours postdose. For other doses, a single ECG will be performed predose. If multiple assessments are due at the same time, the order of priority should be ECG, vitals, PK, and then other assessments, with PK sampling being performed at the nominal time point.

t At Dose 1, the required questionnaires will be performed predose.

u The D169 and EOT FibroScans will be performed prior to the liver biopsies. Participants must be fasting for a minimum of 3 hours prior to the FibroScan being performed.

v Liver biopsy is optional at V19.

w Serious AEs and treatment-emergent SAEs will be assessed until 30 days after the last day of study participation, ongoing SAEs will be followed until resolution or until resolved with sequalae.

x Participants should be fasting for a minimum of 8 hours prior to selected visits.

Table 5: Follow-up Period Schedule of Activities

Post-EOT Week	12	24	36	48[a]	72	96[b]	ET
Window (Days)	± 7	± 7	± 7	± 7	± 7	± 7	N/A
Procedure/Assessment							
DLCO & Spirometry [c]	X	X	X	X	X	X	X
Pulmonary Information [d]	X	X	X	X	X	X	X
Blood biomarkers of fibrosis		X		X	X	X	X
Blood biomarkers of inflammation		X		X	X	X	X
Serum AAT & CRP	X	X	X	X	X	X	X
Hematology/chemistry/coagulation	X	X	X	X	X	X	X
Urine Pregnancy test for WOCBP	X	X					X
UACR & UPCR		X		X		X	X
Serum AFP		X		X	X	X	X
Antidrug antibodies	X			X		X	X
Samples for future analysis				X		X	X
MRE [e]		X		X		X	X
FibroScan® [e]		X		X		X	X
Record AEs/SAEs [f]	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X
Attend visit fasting [g]				X		X	X

Abbreviations: AAT: alpha1 antitrypsin; AFP: alpha fetoprotein; F/U: follow-up; CRP: C-reactive protein; DLCO: diffusion capacity of the lungs for carbon monoxide; EOT: end of treatment; FEV1: forced expiratory volume in one second; FVC: forced vital capacity; MRE: magnetic resonance elastography; SAE: serious adverse event; WOCBP: women of childbearing potential; Z-AAT: the mutant AAT protein.

a The follow-up period for Cohort 3 will terminate at the Week 48 Post-EOT visit. Week 72 and 96 Post-EOT visits will be performed for Cohort 1 and Cohort 2 only.
b If possible, participants prematurely discontinuing from the F/U period will undergo all activities indicated for Week 48 F/U visit.

c Post-bronchodilator values for DLCO and spirometry (FEV_1 and FVC) will be recorded. The order of testing for recorded values should be 1) administer bronchodilator, 2) immediately perform DLCO, 3) perform spirometry.

d Collect and record number and severity of pulmonary exacerbations since the last PFT assessment, resting peripheral oxygen saturation, smoking status including number of cigarettes per day, and use of AAT replacement therapy since the last visit. Assess and record the Modified Medical Research Council dyspnea scale.

e If a clinical site does not have access to the required equipment or technology, MRE assessments would be optional. Participants must be fasting for a minimum of 3 hours prior to the FibroScan or MRE being performed. MRE will only be performed for participants in Cohorts 1 & 2.

f Adverse events/SAEs will be assessed until 30 days after the last day of participation in this study. Ongoing SAEs will be followed until resolution or resolved with sequalae.

g Participants should be fasting for a minimum of 8 hours prior to selected visits.

2. INTRODUCTION

AATD is a rare genetic disease resulting from the abnormal production of the serine protease inhibitor AAT. Normal human AAT is a 52-kDa acute-phase protein that is produced in liver cells. It is released into the plasma in response to inflammatory stimuli. Inherited autosomal mutations in *SERPINA1*, the gene that encodes the AAT protein, leads to the production of a misfolded insoluble protein (Z-AAT) that aggregates in hepatocytes rather than being secreted into the blood (Lomas & Parker, 2006).

AATD is a proteinopathy that affects the lungs and the liver by two different mechanisms. In the lungs, emphysema and COPD are the results of a loss-of-function mechanism, where AAT is not present in adequate amounts to inhibit neutrophil elastase, resulting in the destruction of lung tissue. The liver disease associated with AATD is by a gain-of-toxic function mechanism. The misfolded Z-AAT protein accumulates in the endoplasmic reticulum, leading to hepatocyte cell death and liver inflammation, progressive fibrosis, and, in some cases, hepatocellular cancer (HCC).

More than 100 variants of *SERPINA1* have been described. Approximately 95% of the population of patients with AATD and liver disease express the autosomal homozygous mutant Z-allele (i.e., PiZZ), containing a Glu342Lys missense mutation caused by a GAG to AAG transition mutation in *SERPINA1*. The predicted homozygous PiZZ mutant frequency is approximately 1 in 2000 within northwestern European populations (Blanco et al., 2017; Blanco et al., 2006).

Dicerna Pharmaceuticals, Inc. is developing belcesiran as a treatment for AATLD using an RNAi strategy (McManus and Sharp, 2002). Belcesiran is a sterile solution of the drug substance belcesiran sodium, a synthetic double-stranded RNA oligonucleotide conjugated to GalNAc aminosugar residues, in WFI. The antisense strand of belcesiran sodium is designed to silence the expression of *SERPINA1*. This gene knockdown may lead to a reduction of total Z-AAT expression in the liver and, in turn, reduce the accumulation of the toxic Z-AAT. Without the continuous putative toxic stimulus, the progression of liver disease in affected individuals may be halted, or potentially even reversed.

2.1. Study Rationale

AATLD is a progressive condition resulting in liver fibrosis, and, in some patients, cirrhosis and even HCC. There is no effective treatment for patients with the toxic gain-of-function liver disease associated with AATD, other than standard supportive care, and, in those who progress to end-stage liver disease, organ transplantation. Given the substantial morbidity and mortality of AATLD and the burden of liver transplantation, there is a significant unmet need for a safe and efficacious treatment.

In nonclinical studies, belcesiran sodium (the drug substance for belcesiran) has been shown to significantly reduce target mRNA and AAT protein levels in transgenic mice expressing multiple copies of the human Z-mutant allele of *SERPINA1* and in cynomolgus monkeys expressing wild-type *SERPINA1* at doses of 0.1 mg/kg or greater. Additionally, in the transgenic mouse model of AATLD, belcesiran sodium significantly reduced hepatic globule levels, which is indicative of

reduced accumulation of aggregated human Z-AAT protein. Decreased cellular proliferation and hepatic fibrosis were also observed upon repeated administration of belcesiran sodium. These findings suggest that, by eliminating the toxic protein, belcesiran may prevent and reverse liver disease progression, eliminating the need for liver transplantation. These nonclinical pharmacology studies support the clinical evaluation of belcesiran to treat patients with AATLD.

DCR-A1AT-101 is an ongoing, Phase 1, single-ascending-dose (SAD) study of belcesiran in healthy volunteers. Participants in Study DCR-A1AT-101 received a single dose of DCR-A1AT (0.1, 1.0, 3.0, 6.0, or 12 mg/kg) or placebo and were followed for 56 days (treatment period) thereafter. At the end of the 56 day treatment period, any participant whose serum AAT protein concentration had not returned to ≥ 80% of baseline (or was not within the normal range) returned for PD monitoring visits (conditional follow-up) every 4 (±1) weeks for 2 months, then every 8 (±2) weeks for the next 4 months, and then every 12 (± 3) weeks for an additional 6 months or until AAT returned to ≥ 80% of baseline, whichever is shorter. Currently, all participants have completed the 56-day treatment period and follow-up continues for participants who met the criteria for conditional follow-up.

An interim analysis of PK, PD, and safety data from this single ascending-dose study was used to inform the selection of the dose and dosing interval in this study.

This study is the first evaluation of belcesiran in adults with AATLD.

The overall goals of this study are to:

- demonstrate that belcesiran is safe and well-tolerated in adult PiZZ patients with liver fibrosis;
- investigate the effect of two dose levels of belcesiran on reduction of Z-AAT production in the liver, reflected by a reduction in circulating Z-AAT levels; and
- evaluate the effect of belcesiran on liver disease as assessed by liver biopsy and non-invasive markers of liver disease

2.2. Background

2.2.1. Alpha-1 Antitrypsin Deficiency

Carl-Bertil Laurell and Sten Eriksson first described severe AATD as a condition in which low serum levels of AAT are associated with pulmonary emphysema (Laurell & Eriksson, 1963). The lack of functional AAT in individuals with the PiZZ genotype, in conjunction with other precipitating factors, can lead to unchecked activity of neutrophil elastases in the alveoli, causing emphysema and COPD. In 1969, Harvey Sharp and associates first described cirrhosis in 10 children with AATD (Sharp et al., 1969). In 1971, Sharp described the intrahepatocyte PASD-resistant globules or inclusions, resulting from polymer formation by Z-AAT in the endoplasmic reticulum (Sharp, 1971). In some patients, polymers of Z-AAT can also be detected in the serum using polymer-specific antibodies. Polymers of Z-AAT in serum have been associated with inflammation and pulmonary symptoms and/or more rapid progression of liver disease (Lomas & Parker, 2006; Teckman, 2017).

The natural history of AATD is not well defined. The emergence of clinical liver disease may occur at any age but appears to increase with increasing age in adulthood (Townsend et al.,

2018). Risk factors for the development of liver disease in adulthood include male gender, age over 50 years, repeated elevation in LFTs, hepatitis virus infection, obesity, and metabolic syndrome; although not all factors have been identified in all studies (Elzouki and Eriksson, 1996; Bowlus et al., 2005; Townsend et al., 2018).

The reported prevalence of the clinical liver disease in individuals with AATD varies widely and is likely under-reported. A longitudinal study of 1595 individuals with PiZZ from the Swedish National Patient Register reported a 10% prevalence of the liver disease in adults with PiZZ, with a mean age of liver-disease onset in adults of 61 years (Tanash & Piitulainen, 2019). A 1996 analysis of autopsy data from Sweden detailed that 43% of PiZZ homozygotes had developed cirrhosis, and 28% had HCC (Elzouki and Eriksson, 1996). In the UK, Dawwas et al. reported that 63% of 57 PiZZ homozygotes had a history or clinical findings suggestive of liver disease; or had one or more abnormalities of liver function or liver ultrasound; 17.5% had evidence of severe fibrosis or cirrhosis on biopsy (Dawwas et al., 2013). In a prospective study of liver biopsy data from 94 PiZZ homozygotes in the US and Canada, Clark et al. described that the prevalence of clinically significant liver fibrosis (≥ F2) was 35.1% (Clark et al., 2018). Despite the variability in the reported prevalence of the liver disease in AATD, it is clear that adults with AATD are at significant risk for the development of liver fibrosis, cirrhosis, and HCC.

2.2.2. Pharmacology of Belcesiran

Belcesiran sodium (DCR-S1459, the drug substance for belcesiran) was developed to inhibit the mRNA that encodes the mutated AAT protein. Belcesiran sodium consists of a chemically synthesized double-stranded duplex RNA oligonucleotide conjugated to GalNAc aminosugar residues. After cellular internalization of belcesiran sodium, Dicer protein binds the double-stranded belcesiran sodium molecule and introduces the 22-mer antisense strand into the RNAi pathway in a manner similar to that used by natural miRNAs. The introduction of the belcesiran sodium antisense strand, complementary to *SERPINA1* mRNA, causes selective reduction of *SERPINA1* mRNA and AAT protein expression.

Belcesiran sodium has been shown to significantly reduce target mRNA and AAT protein levels in transgenic mice expressing multiple copies of the human Z-mutant allele of *SERPINA1* and in cynomolgus monkeys expressing wild-type *SERPINA1* at doses of 0.1 mg/kg or greater. Additionally, in the transgenic mouse model of AATLD, belcesiran sodium substantially reduced hepatic globule levels, indicating reduced accumulation of aggregated human Z-AAT protein. Repeated administration of belcesiran sodium in the transgenic mice also demonstrated a decrease in cellular proliferation and hepatic fibrosis.

2.2.3. Nonclinical Pharmacokinetics and Toxicokinetics of Belcesiran

Plasma PK and TK were determined in CD-1 mice, NZW rabbits, and cynomolgus monkeys after SC administration of belcesiran. After a single SC administration, belcesiran was rapidly absorbed into the bloodstream, with a T_{max} of approximately 1 to 2 hours in mice and rabbits and a T_{max} ranging from approximately 2 to 24 hours in monkeys. In mice dosed at 3 mg/kg, C_{max} and AUC_{last} were 115 ng/mL and 155 hr*ng/mL, respectively. In pregnant rabbits dosed at 2 or 6 mg/kg in the definitive EFD study, C_{max} was 252 and 591 ng/mL respectively and $AUC_{0\text{-}48hr}$ was 761 and 2180 hr*ng/mL respectively. In monkeys dosed at 1 mg/kg, C_{max} and AUC_{last} were

122 ng/mL and 1340 hr*ng/mL, respectively, and bioavailability was approximately 30%. In toxicity studies, in mice and monkeys, plasma exposure (C_{max} and AUC_{last}) increased with increasing dose (20 to 300 mg/kg). No sex-related differences were observed in either species and no accumulation in plasma was apparent after up to 7 doses (mice) or 10 doses (monkeys) administered 4 weeks apart in repeat dose toxicity studies. The elimination phase could not be accurately determined in mice. In pregnant rabbits, the estimated $t_{½}$ values ranged from 1.57 to 2.02 hours in the definitive EFD study. In monkeys, mean plasma $t_{½}$ was estimated as 3.2 hours at a dose of 1 mg/kg.

Belcesiran plasma protein binding ranged from 40% to 56% at a concentration of 20 µg/mL in mouse, monkey, and human plasma, with no apparent differences between species.

Distribution of belcesiran following SC administration was primarily to the liver. This finding is consistent with the profile expected for GalNAc-conjugated siRNA that target the hepatocytes via the ASGPR (D'Souza & Devarajan, 2015). In mice, at 24 and 168 hours after a single 3 mg/kg SC dose, belcesiran concentrations in the kidney, which were the next highest concentration, were less than 4% of those in liver. At dose levels of 100 and 300 mg/kg, concentrations in kidney were 7% to 11% of those in liver, and in other organs were less than 2% of those in liver. The mean elimination $t_{½}$ in liver ranged from 3.6 to 4.9 days in mice and was 12 to 17 days in individual monkeys. In toxicity studies, in both CD-1 mice and cynomolgus monkeys, tissue concentrations of belcesiran were consistently higher in the liver than in the kidney 24 hours postdose (mouse) and 48 hours postdose (monkey). In mice and monkeys, the increase in tissue concentrations was less-than-dose proportional in the liver and greater-than-dose proportional in the kidney across the dose range of 20 to 300 mg/kg. Thus, the liver-to-kidney ratio decreased with increasing dose. These results are hypothesized to reflect a dose-related trend toward saturation of hepatocellular uptake via the ASGPR, which in turn increases excretion of the molecule in urine through the kidneys. The greater-than-dose proportional increases in mean C_{max} observed in urine in mice also support this hypothesis.

In pregnant mice, distribution to liver and kidney was similar to that observed in non-pregnant animals. Distribution to fetal liver was observed in mice in the EFD study with doses of 250 to 1000 mg/kg every other day. The concentrations of belcesiran observed in mouse fetal liver were 0.13% or less than that of the dams, indicating limited placental transfer. In pregnant rabbits, distribution to maternal liver and kidney was observed. Belcesiran was observed in fetal plasma and liver, but levels were a fraction of maternal concentrations (< 20% and < 0.0014%, respectively).

Qualitative metabolic profiling was conducted on urine and liver from mice and on urine from monkeys. In mouse liver, the most prevalent antisense strand-related species were FLP and 3′N-1, and the most prevalent sense strand-related species was FLP with the removal of all 4 GalNAc sugars. In mouse and monkey urine, the primary species were FLP and 3′N-1 antisense strand and FLP sense strand.

The interaction of belcesiran with human CYP metabolic enzymes, human ABC (efflux) transporters, and the human SLC (uptake) transporters was evaluated in vitro using human hepatocytes in culture. Data suggest clinically relevant DDI due to transporter inhibition seem unlikely at plasma concentrations up to 37.5 µg/mL and DDI due to CYP enzyme inhibition or induction seem unlikely at plasma concentrations up to 75 µg/mL.

The safety of belcesiran sodium has been characterized in a program of nonclinical studies. Repeat-dose SC toxicity studies of up to 6 months (7 doses) in mice, and up to 9 months (10 doses) in monkeys have been conducted. The 9-month study in monkeys specifically included FDA-requested pulmonary function. Belcesiran sodium has been evaluated for potential genotoxicity in in vitro tests in bacteria and mammalian cells and in vivo in a mouse micronucleus assay. Developmental and reproductive toxicity was assessed in a mouse FEED study and EFD studies in mice and rabbits. The potential for platelet activation by belcesiran sodium was assessed in vitro. In addition, a comprehensive safety pharmacology study was conducted.

Overall, belcesiran is well tolerated following chronic administration to mice and monkeys at dose levels up to 300 mg/kg. In monkeys, no adverse on-target effects were observed despite the persistent and almost complete knockdown of *SERPINA1* mRNA in the liver. No toxicity indicative of off-target hybridization was evident in mice or monkeys. Non-adverse test article related effects were limited primarily to those reported with other oligonucleotides including those that are GalNAc conjugated (e.g., basophilic granules in hepatocytes and vacuolated macrophages) and were not degenerative in nature (Henry et al., 1999; Janas et al., 2018; EMA/70703/2020; EMA/568312/2020). Of note, minimal vascular inflammation of the pulmonary arteries and other tissues observed in the 3-month study was not recapitulated in the 9-month monkey study. Further, in the 9-month monkey study, there were no belcesiran-related effects on pulmonary function testing (lung diffusion capacity for carbon monoxide, quasi-static lung compliance, and various indices derived from the flow-volume loop, including FVC and FEVt, and flow rates [FEFv]) and no histologic evidence of adverse pulmonary effects such as those reported in PiZZ patients.

Belcesiran was negative in the bacterial reverse mutation assay and did not induce micronuclei in mammalian cells in vitro. There were no adverse effects on CV, respiratory, and neurological function in in vitro or in vivo studies with belcesiran. Administration of belcesiran was not associated with any effects typically attributable to oligonucleotide chemical modifications. Belcesiran was not associated with adverse injection site reactions in either species.

Belcesiran and its active mouse analog (DCR-m0641) did not affect maternal viability or reproductive function in a mouse FEED study (NOAEL was 1000 mg/kg, the highest dose of belcesiran administered). In the mouse EFD study, modest (~5% to 6%) reductions of embryo-fetal weights were observed. There were no increases in malformations in any dose group. The fetal weight effect was observed in both belcesiran and mouse-active analog groups and occurred in the absence of on-target pharmacologic activity, as there was no *Serpina1* mRNA reduction in the fetuses from dams administered the active analog. Belcesiran was detected in mouse fetal liver at 0.13% or less than that of the dams, indicating limited placental transfer. The developmental NOAEL was 500 mg/kg/dose (total dose 2500 mg/kg), which is > 65 x greater than the monthly human dose (3 mg/kg) on a mg/kg basis. The human relevance of the decreased fetal weights is unclear, especially in the absence of target mRNA reduction and negligible fetal exposure.

In the rabbit EFD studies, repeat and single dose (DRF EFD study) administration of belcesiran during organogenesis resulted in maternal weight loss and lower food consumption that began within 24 hours of the first dose and was of particular note throughout the first few doses. Higher post-implantation loss and lower numbers of live fetuses, and increased external, visceral, and/or

skeletal malformations were observed after repeat or single doses of belcesiran. Belcesiran exposure in fetal plasma after repeated dosing of 10 or 50 mg/kg/dose (DRF study) and in fetal liver at 50 mg/kg/dose was observed but were a fraction of maternal concentrations and belcesiran was not detectable after repeat-dosing of 0.2 to ≤6 mg/kg. These data suggest that placental transfer is negligible at dose levels similar to those administered in human clinical trials. In the definitive EFD study, a dose level of 2 mg/kg/dose was considered to be the NOAEL for maternal toxicity and based on postimplantation loss noted at 6 mg/kg/dose and visceral malformations of the heart and major blood vessels noted at ≥ 0.6 mg/kg/dose, a dose level of 0.2 mg/kg/dose was considered to be the NOAEL for embryo-fetal development.

Of note, belcesiran does not have complete homology with the rabbit A1AT mRNA sequence (7 mismatches) and therefore is not predicted to be pharmacologically active in the rabbit. In an exploratory EFD study (data available upon request), single and repeat dosing of a pharmacologically inactive but chemically comparable GalNAc-conjugated siRNA demonstrated similar maternal effects on food consumption, body weights, and post-implantation loss (skeletal and visceral effects not characterized). A lack of pharmacologic activity of belcesiran in rabbits and similar effects with the inactive but chemically comparable GalNAc-conjugated siRNA demonstrate that the belcesiran-related EFD effects are associated with the bulk chemical and physical properties (e.g., high molecular weight, polyanionic polymer) of the molecule rather than pharmacology (i.e., RNAi). The maternal body weight loss and decreased food consumption in conjunction with no measurable fetal exposure, are evidence of maternal effects driving the EFD effects rather than a direct effect on the fetus. The human relevance of the EFD effects in rabbits is unclear, as similar toxicities were not observed in mice with belcesiran nor the pharmacologically active mouse analog.

2.2.4. Clinical Overview

Belcesiran is currently being evaluated in one ongoing, Phase 1 study in healthy volunteers. Study DCR-A1AT-101 is a multi-cohort, randomized, placebo-controlled, SAD study evaluating the safety, tolerability, PK, and PD of belcesiran at doses ranging from 0.1 to 12.0 mg/kg (sodium salt). Participants are followed for at least 56 days after single dose administration on Day 1. Participants whose serum AAT protein concentration has not returned to ≥ 80% of baseline (or is not within normal range) at Day 57 every 4 (± 1) weeks for 2 months, then every 8 (± 2) weeks for the next 4 months, and then every 12 (± 3) weeks for an additional 6 months or until AAT has returned to ≥ 80% of baseline, whichever is shorter.

All cohorts (0.1, 1.0, 3.0, 6.0, and 12.0 mg/kg) in Study DCR-A1AT-101 are complete through Day 57 (the EOT visit), and all eligible participants are in C-F/U.

No SAEs, severe TEAEs, or DLTs have been reported. All TEAEs were mild in severity except for 3 events of moderate severity reported for 2 participants: nasopharyngitis (1 participant in Cohort 1, 0.1 mg/kg belcesiran), gastroenteritis and staphylococcal skin infection (1 participant in Cohort 4, 6.0 mg/kg belcesiran).

Thus far, the medical monitor and SRC have not identified any safety concerns.

Belcesiran, at single doses of 1.0 mg/kg, 3.0, 6.0 and 12.0 mg/kg, resulted in robust reductions in serum AAT concentrations. The mean reduction in serum AAT concentration at the EOT visit ranged from approximately 48% for the 1.0 mg/kg cohort to 78% for the 12.0 mg/kg cohort.

2.2.5. Summary

Adults with AATD are at significant risk for the development of progressive liver disease. Due to the variable natural progression of the disease, it is currently not possible to predict which adults with AATD will remain stable, and which will develop life-threatening liver disease over time. Some patients with progressive liver fibrosis or compensated liver cirrhosis can remain stable for many years with little need for intervention, while others may progress to fulminant liver failure and death.

While augmentation therapy may address the loss of AAT in the lungs, no treatment yet exists for the toxic gain-of-function-mediated liver disease. The knockdown of *SERPINA1* mRNA should lead to a reduction of total Z-AAT expression in the liver, which in turn reduces the accumulation of the toxic Z-AAT protein. Without the continuous putative toxic stimulus, the progression of liver disease in affected individuals may be halted, or potentially even reversed.

Nonclinical studies of belcesiran sodium in transgenic mice and cynomolgus monkeys have shown a significant reduction of target mRNA and AAT protein levels. In transgenic mice, belcesiran sodium substantially reduced hepatic globule levels, indicating reduced accumulation of aggregated human Z-AAT protein.

The clinical evaluation of belcesiran is ongoing. The initial safety, tolerability, PK, and PD of belcesiran are being evaluated in a Phase 1 SAD study in healthy volunteers (DCR-A1AT-101). To date, belcesiran administration in healthy volunteers appears to be safe and well-tolerated, with no SAEs or DLTs reported. The SRC for Study DCR-A1AT-101 and the DSMC from this study have reviewed all available safety data from all cohorts and have affirmed that it is safe to advance the proposed dosing regimens in this study. Two belcesiran dose levels (210 mg and 50 mg) will be assessed in this study. The doses selected span a range of expected serum AAT reduction, allowing for assessment of the dose-exposure-response relationship of belcesiran and its safety profile with different levels of AAT knockdown (see Section 4.3 for further details).

Study DCR-A1AT-201 is designed to demonstrate the safety and tolerability of belcesiran in adults with AATLD and to establish proof-of-concept that belcesiran can reduce Z-AAT production, as reflected by a reduction in circulating Z-AAT levels. Paired liver biopsies will be employed to evaluate levels of the mutated Z-AAT protein and its polymers in the liver and the degree of liver fibrosis over time.

2.3. Benefit/Risk Assessment

2.3.1. Potential Risks Associated with Belcesiran

The summary of potential risks provided below is based on an in-depth review of literature relating to the proposed approach, and insights from the nonclinical pharmacology, toxicology studies and human Phase 1 data.

Potential safety issues that may be associated with belcesiran can be characterized into 3 broad categories: risks associated with the siRNA molecule, risks associated with the knockdown of AAT, and risks associated with potential off-target effects.

2.3.1.1. Risks Related to the siRNA Molecule

Due to the lack of excipients in the drug product, the potential risks associated with belcesiran are limited to the risks associated with the siRNA oligonucleotide portion of the drug product (belcesiran sodium).

To date, belcesiran sodium has been associated with little-to-no toxicity in the nonclinical studies and no toxicity in a Phase 1 study in healthy volunteers. Results of nonclinical toxicity studies of belcesiran sodium conducted to date are presented in Section 2.2.3.

Clinical experience with modern GalNAc siRNA platforms, including the Sponsor's GalXC platform, suggest an enhanced safety profile with no evidence for systemic immune stimulation or toxicities observed in more than 150 humans dosed with GalNAc siRNAs (Dicerna data on file).

Nevertheless, all study participants will be monitored for signs or symptoms of systemic toxicity, decline in liver or kidney function, abnormalities on hematologic parameters (including platelets and abnormalities in coagulation parameters), and abnormalities in vital signs, ECGs, or other relevant safety parameters.

2.3.1.2. Risks Related to AAT Knockdown

There is currently little experience with AAT knockdown in patients with PiZZ AATLD. However, the knockdown of AAT could potentially worsen the related loss-of-function effects in the lungs, since it has been shown that subjects with Null mutations of the *SERPINA1* gene and thereby no Z-AAT production, are at a particularly high risk of emphysema. It is however important to note that the pulmonary manifestations in the Null mutation population usually develop after decades of total absence of the AAT protein (Fregonese et al, 2008).

In the DCR-A1AT-101 study, a mean knockdown of 78% was achieved and no clinically significant changes in lung function were noted on spirometry or DLCO, no dose-dependent changes were noted in repeat measurements of any spirometry parameter, and all Percent Predicted DLCO measurements remained within normal limits across all cohorts.

In addition, others have reported that no clinically meaningful changes in spirometry were observed after 40 weeks (N=16) or 72 weeks (N=4) of Z-AAT knockdown in patients with AATLD (Strnad et al., 2021).

To mitigate this risk, serial measurements of PFTs will be conducted throughout the study, and whenever applicable augmentation therapy will be able to restore absent serum AAT protein concentrations and anti-neutrophil elastase capacity to levels at the lower limit of normal (Wewers, 1987; Sandhaus et al., 2016; Chapman et al., 2015; Sorrells et al., 2015).

In study participants already on augmentation therapy at the initiation of the study, the risk of worsening the loss-of-function disease mechanism in the lungs is reduced because these study participants have non-mutated M-AAT levels restored to the lower limit of normal and this would not be affected by the Z-AAT knockdown (Chapman KR, Chorostowska-Wynimko J, Koczulla AR, Ferrarotti I, McElvany NG. Alpha 1 antitrypsin to treat lung disease in alpha 1 antitrypsin deficiency recent developments and clinical implications. Int J Chron Obstruct Pulmon Dis. 2018;13:419-432.

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2.3.1.3. Risks Related to Potential Off-Target Effects

In silico analysis for the potential for off-target activity of belcesiran was assessed via thorough bioinformatics analysis that suggests it has minimal potential for off-target effects via siRNA hybridization to the human genome. Potential off-target homologies at the levels > 15 contiguous NTs were completely absent from the human transcripts analyzed other than *SERPINA1*. Such a gap suggests excellent specificity for the intended target *SERPINA1*. Only four genes (*KLKB1*, *DAPK1*, *CAMLG*, and *KATNAL1*) with seed region complementarity and moderate to high expression in the liver were identified. Additionally, in an in vitro assay in a human hepatocellular carcinoma cell line, the IC50 for belcesiran against its target transcript SERPINA1 was approximately 0.09 pM, which is > 18,000-fold lower than the genes identified with partial complementarity to the antisense strand of belcesiran (*KLKB1*, *DAPK1*, *CAMLG*, and *KATNAL1*) that would be expressed in normal liver. This indicates that none of the selected genes with partial complementarity to the antisense strand of belcesiran are likely to be functionally reduced in expression by treatment with belcesiran. These findings, combined with the high specificity of GalNAc conjugates for the delivery of belcesiran to the liver suggest a low likelihood of hybridization based off-target effects for belcesiran. Overall, these results suggest that the belcesiran sequence has a high degree of specificity for the intended *SERPINA1* mRNA target with minimal potential to exert unintended RNAi effects against nontarget genes. The potential risks associated with off-target effects remain only hypothetical because of the lack of complementarity, and the results of the in vitro studies demonstrate that belcesiran has extremely low activity with the genes tested. Additionally, in the Phase 1 study of belcesiran in healthy volunteers, there was no evidence of a patient safety signal associated with these genes. Overall, there is no evidence of patient risk from these potential off-target effects.

2.3.2. Risks Associated with Liver Biopsy and Study-Specific Evaluations

To be eligible for participation in the study, prospective participants must agree to undergo 2 liver biopsies.

The Screening liver biopsy will only be conducted once the prospective participant has met all other eligibility criteria.

Percutaneous liver biopsy is a standard diagnostic procedure that bears several risks. Milder and more common risks include pain and bruising at the biopsy or incision site. More serious complications include prolonged bleeding from the biopsy or incision site or internal bleeding (which may require hospitalization, transfusions, and sometimes surgery or another procedure to stop the bleeding), infection of the biopsy site or incision site that may cause sepsis, and pneumothorax, hemothorax, or puncture of other organs (Liver Biopsy-NIDDK). The overall risk of serious complications following liver biopsy is approximately 1% (Boyum et al., 2016), and the risk of death directly related to the liver biopsy is approximately 1 in 10,000 (West & Card, 2010).

Liver biopsy may be conducted under procedural sedation and analgesia (PSA) to reduce potential pain and anxiety for the study participant. PSA bears a low risk of complications but may include respiratory compromise with resultant hypoxia or hypercarbia, vomiting, aspiration,

hypotension, bradycardia, and drug reaction. The overall incidence of SAEs during PSA, such as laryngospasm, aspiration, and intubation, is rare (Bellolio et al., 2016).

The risks associated with the liver biopsies are minimized through the selection of suitable study participants through eligibility criteria and safety precautions outlined in detail in the Liver Biopsy Manual.

Study-specific evaluations and sampling procedures, e.g., blood-pressure measurements using a blood pressure cuff and frequent blood-sampling, may cause transient discomfort and carry a low risk of bleeding, hematoma formation, peripheral nerve damage, or infection.

2.3.3. Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Intervention		
AEs and SAEs	This is the first multiple dose study of belcesiran in humans and the first evaluation of belcesiran in adults with AATLD. All AEs are considered to be unexpected. In the ongoing first-in-human study (DCR-A1AT-101), belcesiran has been well tolerated. No SAEs or dose-limiting toxicities have been reported. Thus far, the medical monitor and SRC have identified no safety concerns. The SRC has endorsed the expansion of each cohort beyond the sentinel pair and each proposed dose escalation. The DSMC reviewed all available data from DCR-A1AT-101 prior to dosing of the first participant in this study (DCR-A1AT-201) and found no safety concerns.	Both proposed belcesiran fixed doses (210 mg and 50 mg) are within the dose range tested in the DCR-A1AT-101 study (with 210 mg corresponding approximately to a 3 mg/kg dose level) and are expected to be safe and well-tolerated. The highest dose tested in DCR-A1AT-101 is 12 mg/kg. The DSMC has reviewed a minimum of 3 weeks of data from a sentinel pair of participants in this DCR-A1AT-201 and allowed for continued enrollment of participants. The DSMC will continue to review data throughout the study, as per the DSMC charter.
Oligonucleotide class risks, including stimulation of pattern recognition receptors leading to cytokine release, inflammation, injection site reactions, low elevations of LFTs, and a prolonged aPTT	The various RNA and DNA chemical modifications studied over 3 decades of clinical trials of ASOs, with and without phosphorothioates, suggest these class toxicities are highly dependent on phosphorothioate modifications. Single- or double-stranded oligonucleotides delivered in lipid nanoparticles, major adverse reactions include infusion reaction symptoms typical of other lipid-particle-based drugs.	GalNAc-siRNA conjugates have chemical modifications to resist enzymatic degradation, reduce or eliminate cytokine responses, reduce non-specific protein binding (the source of coagulopathies), and target the siRNAs to the hepatocytes, thus eliminating the need for any lipid nanoparticle formulation. Participants will be monitored for signs or symptoms of systemic toxicity, decline in liver or kidney function, abnormalities in hematologic parameters (including platelets and abnormalities in coagulation parameters), and abnormalities in vital signs, ECGs, or other relevant safety parameters

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
AAT knockdown	An average of approximately 80% reduction from baseline Z-AAT could potentially worsen the loss-of-function disease mechanism in the lungs in patients who are not receiving augmentation. In this patient population, an average of approximately 80% reduction from baseline Z-AAT knockdown of Z-AAT in the liver would result in a nearly complete absence of Z-AAT protein in the blood. Subjects with Null mutations, which are also devoid of AAT production, are at a particularly high risk of emphysema; however, these pulmonary manifestations usually develop after decades of total absence of the AAT protein (Fregonese et al, 2008).	Only participants with stable lung function are eligible for enrollment, including participants who may be on augmentation therapy. Serial measurements of PFTs will be conducted throughout the study. Augmentation therapy to restore serum AAT protein concentrations can be provided.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
SC injection	The study drug is administered via SC injection in the abdomen or thigh. Subcutaneous injection is associated with pain and may cause vasovagal reactions, allergic reactions, infections, and bleeding.	The risk/burden associated with the SC injection of the study drug is minimal and will be controlled by close monitoring of the events. Therefore, SC injections are assessed as a minimal risk/burden to patients.
Blood sampling, including cannulation	Risks include acute pain, bleeding, vessel injury, and, in rare instances, arterial vessel blockage, potentially leading to infection.	The risk/burden is minimal and not over and above the risk/burden associated with blood sampling as part of the standard treatment for AATLD. Due to the increased frequency of blood sampling in the trial to ensure patient safety, the likelihood of patients experiencing minor effects from blood sampling may be higher during the study.
Biopsy	Percutaneous liver biopsy is a standard invasive diagnostic procedure that bears several risks. Milder and more common risks include pain and bruising at the biopsy or incision site. More serious complications include prolonged bleeding from the biopsy or incision site, internal bleeding (which may require hospitalization, transfusion, and sometimes surgery or another procedure to stop the bleeding), infection of the biopsy site or incision site that may cause sepsis, and pneumothorax, hemothorax, or puncture of other organs. The overall risk of serious complications following liver biopsy is approximately 1% (Boyum et al., 2016).	The risks associated with the liver biopsies are minimized through the selection of suitable study participants through eligibility criteria and safety precautions outlined in detail in the Liver Biopsy Manual. Safety precautions include monitoring of the patient during the procedure and post procedure per local standard of care. The low risk of respiratory compromise in the elective setting of PSA is minimized through the standard-of-care monitoring and the presence of a healthcare provider with the knowledge and skills to recognize and treat airway complications.

Abbreviations: AAT: alpha 1 anti-trypsin; AATLD: Alpha-1 Antitrypsin Deficiency-Associated Liver Disease; AE: adverse event; aPTT: activated partial thromboplastin time; ASO: antisense oligonucleotide; DNA: deoxyribonucleic acid; DSMC: Data Safety Monitoring Committee; ECG: electrocardiogram; GalNAc: *N*-acetylgalactosamine; LFT: liver function tests; PFT: pulmonary function test; PSA: procedural sedation and anesthesia; RNA: ribonucleic acid; SAE: serious adverse event; SC: subcutaneous; siRNA: small interfering ribonucleic acid; *SERPINA1:* the mutant AAT protein; SRC: Safety Review Committee; Z-AAT: the mutant AAT protein.

2.3.4. Overall Benefit: Risk Conclusion

At present, no pharmacological therapies are approved for the treatment of patients with AATLD. These patients are at increased risk of developing cirrhosis and portal hypertension (PHT). Complications associated with PHT include gastrointestinal bleeding, ascites, encephalopathy, and reduced levels of platelets, or decreased white blood cell count. The standard-of-care includes the use of vasoactive drugs, endoscopic band ligation, and antibiotic prophylaxis. Patients failing standard-of-care treatment may undergo transjugular intra hepatic portosystemic shunt (TIPS) placement. Ultimately, the only cure for AATLD is liver transplantation.

Belcesiran has been shown to decrease serum AAT protein concentrations in healthy volunteers. In Study DCR-A1AT-101, the mean reduction in serum AAT concentration at EOT visit ranged from approximately 48% for the 1.0 mg/kg cohort to 78% for the 12.0 mg/kg cohort. Belcesiran treatment has the potential benefit to reduce the production and, hence, the accumulation of the misfolded Z-AAT protein in the liver. By eliminating the toxic protein, belcesiran may prevent liver disease progression, which ultimately may eliminate the need for liver transplantation.

Participants with AATLD could potentially benefit from the investigational drug. This potential benefit has not been proven and may be limited in duration to the treatment period of this trial.

The key potential risks associated with belcesiran include exacerbation of emphysema, a decline in lung function, elevations of LFTs, and injection site reactions. All these risks are monitorable and should be reversible over time after drug discontinuation or could potentially be treated with intravenous augmentation therapy in the case of knockdown-mediated lung function decline. In DCR-A1AT-101, single doses of belcesiran in healthy volunteers (up to and including 12 mg/kg) appeared to be safe and well tolerated. Repeat measurements of spirometry and DLCO indicated that normal lung function was preserved. No clinically significant or apparent dose-dependent increases in AST or ALT levels were observed.

In study participants already on augmentation therapy at the initiation of the study, the risk of lung function decline due to reduction of Z-AAT serum levels would be reduced because these study participants have non-mutated M-AAT levels restored to the lower limit of normal.

The primary potential risk associated with percutaneous liver biopsy in participants with AATLD is bleeding. This risk is minimized through the selection of suitable participants through eligibility criteria that exclude participants with decompensated cirrhosis (Child Pugh Score B or C) and by the safety precautions outlined in the Liver Biopsy Manual. The low risk of respiratory compromise in the elective setting of PSA is minimized through the standard of care monitoring and the presence of a healthcare provider with the knowledge and skills to recognize and treat airway complications.

In summary, the Sponsor believes the Benefit to Risk ratio to be favorable. Ongoing risk-benefit assessments will be conducted throughout the trial by the independent DSMC.

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary (Cohorts 1 and 2)	
1. To evaluate the safety and tolerability of multiple doses of belcesiran in patients with AATLD	1. The incidence and nature of TEAEs, and the change from Baseline in PFTs, 12 -lead ECGs, physical examination findings, vital signs, and clinical laboratory tests
2. To characterize the PD of belcesiran in patients with AATLD	2. Changes from baseline to weeks 24 (Cohort 1)/48 (Cohort 2) in serum AAT protein concentrations
Primary (Cohort 3)	
3. To characterize the PD of belcesiran in patients with AATLD	3. Change from baseline to week 24 in serum Z-AAT protein levels 4. Change from baseline to week 24 in liver Z-AAT protein levels
Secondary	
1. To characterize the PK of belcesiran in the plasma of patients with AATLD	1. PK profile of belcesiran
2. To assess the effect of belcesiran on liver histology in patients with AATLD	2. Change from Baseline up until week 96 in liver fibrosis 3. Change from Baseline up until week 96 in diastase-resistant PAS-positive AAT globules
Exploratory	
1. To assess the effect of belcesiran on liver stiffness in patients with AATLD	1. Change from Baseline up until week 96 in FibroScan® score
2. To assess the effect of belcesiran on liver fibrosis and/or inflammation in patients with AATLD	2. Change from Baseline up until week 96 in ELF score 3. Change from Baseline up until week 96 in CK-18

4. STUDY DESIGN

4.1. Overall Study Design

This study is a multiple dose, randomized, placebo-controlled, double-blind study to evaluate the safety, tolerability, PK, and PD of 2 doses of belcesiran in adult patients with PiZZ AATLD (liver fibrosis F1, F2, F3, or F4, METAVIR scoring system).

After the screening period, participants will be randomized, and administration of IMP (investigational medicinal product) will be initiated. Participants will return to the site for administration of IMP and safety, tolerability, PK, and PD monitoring at specified time points as per the SoA through the EOT visit. If participants are not able to return to the site for assessments or procedures, visits may be conducted by qualified medical professionals as at-home telemedicine and/or home nursing visits, at the discretion of the Investigator.

The study will be conducted in 3 separate cohorts. Participants will be randomized in a 1:1 fashion to either Cohort 1 and 2. Once Cohort 1 and 2 have enrolled, enrollment of Cohort 3 will be initiated.

- Participants in Cohort 1 (N=8) will be randomized 3:1 to either belcesiran 210 mg or placebo and a liver biopsy will be performed at week 24 to assess the effect of belcesiran. In addition, participants will have the option to continue treatment for an additional 72 weeks so that the total treatment duration will be 96 weeks.
- Participants in Cohort 2 (N=8) will be randomized 3:1 to either belcesiran 210 mg or placebo and a liver biopsy will be performed at week 48 to assess the effect of belcesiran. In addition, participants will have the option to continue treatment for an additional 48 weeks so that the total treatment duration will be 96 weeks.
- Participants in Cohort 3 (N=30) will be randomized 2:1:2:1 to belcesiran 210 mg, the equivalent amount of placebo for belcesiran 210 mg, belcesiran 50 mg or the equivalent amount of placebo for belcesiran 50 mg. Participants will be blinded within each dose level. Participants will have a liver biopsy performed at week 24 to assess the effect of belcesiran and thereafter continue treatment until 96 weeks of treatment have been completed.

Randomization will be stratified based on fibrosis stage (METAVIR Score F1/F2, F3, or F4) in all cohorts.

Participants in Cohort 1 and 3 will receive monthly dosing for the first 24 weeks and then shift to quarterly dosing thereafter until week 96. Participants in Cohort 2 will receive monthly dosing for the first 48 weeks and then shift to quarterly dosing thereafter until week 96. Participants will continue the IMP throughout the study, unless the participant or investigator decide to discontinue IMP, or the participant withdraws from the trial or a discontinuation criterion is met.

All Participants in Cohorts 1 to 3 will have the option to undergo a liver biopsy at EOT/week 96. The biopsy at 96 weeks will inform about Z-AAT levels and provide further information about possible improvements in liver histology. Biopsy findings will be correlated with changes in non-invasive markers of liver disease and liver stiffness.

After the EOT visit, all participants will be followed up for 48 weeks. In addition, Cohorts 1 and 2 will be followed for an additional 48 weeks to inform about the duration of potential treatment

effects. If a participant in Cohorts 1 or 2 does not wish to extend the treatment period to 96 weeks, follow-up will stop after the 48-week follow-up period.

There are 3 interim analyses planned for this trial. Interim analyses for Cohorts 1 and 2 will take place after all participants in Cohorts 1 and 2 complete 24 weeks and 48 weeks of treatment, respectively. The third interim analysis will be performed when all the participants in Cohort 3 have completed 24 weeks of treatment. The third interim analysis will evaluate the dose-response relationship on efficacy and safety assessments of the two doses of belcesiran. If based on the interim analysis it is concluded that one dose is more favorable with respect to effect while showing no overall difference in safety – the least favorable dose-arm can be stopped.

The trial will be monitored by an independent external DSMC. The DSMC will review accumulating data from the trial in an unblinded fashion to ensure adequate monitoring of safety. The timing and scope of the review meetings will be defined in the DSMC charter.

4.2. Scientific Rationale for Study Design

As this is the first evaluation of belcesiran in patients with AATLD, safety and tolerability are best assessed via a double-blind, placebo-controlled study. Because AATLD is a rare disease, the use of an unbalanced randomization ratio will minimize exposure of participants to placebo whilst still allowing for an unbiased assessment of safety and maximizing the amount of PD data to be collected.

It is expected that the knockdown of *SERPINA1* mRNA will result in decreased production of Z-AAT in the liver, and hence decreased levels of circulating AAT in serum.

The effect of belcesiran on AATLD will be assessed via changes in the levels of Z-AAT in the liver and serum, as well as by changes in liver histology. Liver biopsy is regarded as the gold standard for the direct assessment of the progression of liver disease over time. The use of 3 parallel cohorts with biopsies scheduled at either weeks 24 or 48 as well as the optional biopsy at week 96 will allow for the evaluation of treatment effect at different timepoints especially since change in liver histology might require a longer treatment period. Biopsy findings will be correlated with changes in non-invasive markers of liver disease and will potentially be supported by “omics” analyses, such as proteomics.

The targeted population includes patients with fibrotic liver disease who are most likely to benefit from a future therapy with belcesiran, and excludes patients with liver impairment or decompensated cirrhosis, as these patients are at higher risk for complications, including those associated with liver biopsy. WOCBP are eligible if they agree to follow the contraception guidelines provided in Section 10.4.2.

4.3. Justification for Dose

Two doses of belcesiran will be evaluated in this study: 210 mg and 50 mg. No safety concerns have been identified by the SRC or DSMC who reviewed all available DCR-A1AT-101 data prior to dosing the first patient in DCR-A1AT-201. Both doses selected for this study are within the dose range tested in DCR-A1AT-101 and are expected to be safe and well-tolerated. In addition, the NOAEL plasma exposure observed in the pivotal repeat-dose toxicity studies provides adequate safety margin for the expected exposure at these doses (at least 23-fold to the

high dose proposed in this study). Details on clinical safety data from DCR-A1AT-101 and nonclinical toxicology studies are provided in the DCR-A1AT Investigator Brochure.

A PK/PD modeling and simulation analysis was performed using all available PK and PD (serum AAT protein levels) data from DCR-A1AT-101 including data up to EOT for 12 mg/kg dose and all available data from C-F/U period. The adequacy of models in describing the available data and the predictive performance of the final model were assessed using pre-specified goodness-of-fit criteria. Based on the final PK/PD model, simulations of serum AAT profiles were performed to inform the dosing regimen for this study. A dataset of 1000 virtual adult subjects was created by sampling the demographics for these virtual subjects from the National Health and Nutrition Examination Survey (NHANES; http://wwwn.cdc.gov/nchs/nhanes/search/nhanes_continuous.aspx.), with age range of 18 to 65 years and weight range of 41 to 199 kg (median weight of 77.5 kg). Various dosing regimens, including weight-based dose and fixed dose with different dosing intervals, were evaluated. The simulations over the wide range of weight indicated that weight-based and equivalent fixed-dose regimens would result in similar reductions in serum AAT. Given similar predicted PD responses, a fixed-dose regimen is preferred over the weight-based regimen for ease in dose administration.

The 210 mg q4w dosing regimen was selected to target a near-maximal PD response. From the simulations, the mean reduction in serum AAT was predicted to be approximately 82% by Week 24 (90% prediction interval [PI]: -59.1% to -98.8%). A lower dose of 50 mg q4w was included to better characterize the dose-exposure-response relationship of belcesiran and its safety profile over a range of serum AAT reduction. Following the administration of 50 mg q4w, the mean reduction in serum AAT was predicted to be approximately 65% (90% PI: -44.5% to -82.4%) by Week 24. Simulations predicted a serum AAT reduction of at least 50% by Week 24 in about 90% of the participants receiving 50 mg q4w dose, suggesting a 50% reduction in hepatic AAT production. It has been shown that adults with the *SERPINA1* PiMZ genotype, which leads to an approximately 50% reduction in hepatic Z-AAT production, have lower levels of serum transaminases, fewer AAT inclusions in liver, and lower liver stiffness than adults with the PiZZ genotype, although higher than adults without the PiZ variant (Schneider et al, 2020). Therefore, the 50 mg q4w dose, which is predicted to reduce hepatic Z-AAT production (and hence the downstream serum Z-AAT) by at least 50% in the majority of study participants with AATLD, may offer some clinical benefits to most of the participants receiving this dose while expanding the range of PD response for dose-exposure-response characterization.

Monthly dose frequency has been selected for Cohorts 1 and 2 to maximize potential PD response. The first 6 months dosing in Cohort 3 will also be monthly after which patients will shift to quarterly dosing. The rationale for exploring an alternative dosing regime is out of consideration for patient burden. For the high dose explored, simulations suggest that reducing dose frequency after steady state would only have a minor effect on the PD response.

In summary, the two belcesiran doses included in the clinical protocol (210 mg and 50 mg) are predicted to result in a range of PD response that would allow for adequate characterization of belcesiran dose-exposure-response in patients with AATLD.

4.4. End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study (including F/U), including the last visit or the final scheduled procedure shown in the SoAs (Section 1.3).

The end of the study is defined as the completion of the final database lock.

5. STUDY POPULATION

5.1. Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age

1. Age 18 to 75 years, inclusive, at the time of signing the ICF.

Type of Participant and Disease Characteristics

2. Documented diagnosis of PiZZ-type AATD, confirmed by genotyping. Historical genotyping data may be used, if available.
3. AATLD, with a liver fibrosis score categorized as F1, F2, F3, or F4 in the METAVIR scoring system, documented by liver biopsy during Screening.
4. Post-bronchodilator FEV_1 > 45% of predicted at Screening.
5. Participants receiving augmentation therapy on a regular basis and intending to continue augmentation therapy during the study are eligible to participate.
6. eGFR at Screening ≥ 60 mL/min normalized to 1.73 m^2 BSA.
7. Non-smokers (defined as having not smoked cigarettes daily for at least the preceding 12 months) with current non-smoking status confirmed by urine cotinine at Screening AND any previous smoking history prior to 12 months must be < 15 pack years, including use of e-cigarettes. Participants may be on nicotine replacement (patch or gum). A positive urine cotinine result due to nicotine replacement is acceptable for enrollment at the discretion of the Investigator.

Sex

8. Male or female

   Male participants:

   A male participant with a partner of childbearing potential must agree to use contraception, as detailed in Section 10.4.2, during the treatment period and for at least 12 weeks after the last dose of study intervention and refrain from donating sperm during this period.

   Female participants:

   A female participant is eligible to participate if she is not pregnant (see Section 10.4.2), not breastfeeding, and at least one of the following conditions applies:

Not a woman of childbearing potential (WOCBP) as defined in Section 10.4.1

OR

A WOCBP who agrees to follow the contraceptive guidance in Section 10.4.2 during the treatment period and for at least 12 weeks after the last dose of study intervention.

Contraceptive use should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

Informed Consent

9. Capable of giving signed informed consent, which includes compliance with the requirements (including consent to undergo paired liver biopsies) and restrictions listed in the ICF and in this protocol.

5.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Any condition which, in the investigator's opinion might jeopardize participant's safety or compliance with the protocol.
2. History of chronic liver disease other than non-alcoholic fatty liver disease from any cause other than PiZZ-type AATD. Diagnostic testing exclusions are defined in the Diagnostic Assessments section below.
3. Child-Pugh Score B or C or a previous hepatic decompensation event.
4. History of one single severe exacerbation of underlying lung disease in the year prior to randomization.

   A severe exacerbation is defined as an exacerbation that requires hospitalization or a visit to the emergency room.

5. History of rapid decline in pulmonary function, as assessed by the Investigator.
6. Known or suspected abuse of drugs in the opinion of the Investigator.
7. Known or suspected excessive consumption of alcohol (≥ 21 units of alcohol per week in men and ≥ 14 units of alcohol per week in women; where a "unit" of alcohol is equivalent to a 12-ounce beer, 4-ounce glass of wine, or 1 ounce shot of hard liquor as defined by the World Health Organization).
8. Any of the following: myocardial infarction, stroke, classification of heart failure New York Heart Association (NYHA) Class IV, hospitalization for unstable angina pectoris or transient ischemic attack within the past 90 days prior to the day of screening (V2A) and between screening and randomization.
9. History of malignancy, unless the malignancy (other than hepatocellular or lung cancer) has been in complete remission off chemotherapy and without additional medical or surgical

interventions within the preceding 5 years, or unless the malignancy has been an adequately treated skin cancer (other than melanoma) or, superficial bladder tumor, or in situ cervical cancer in the preceding 1 year.

Prior/Concomitant Therapy

5. Use of an RNAi drug at any time.
6. History of one or more of the following reactions to an oligonucleotide-based therapy:
    a. severe thrombocytopenia (platelet count < 100,000/ mm^3)
    b. hepatotoxicity, defined as ALT or AST > 3 × ULN and total bilirubin > 2 × ULN or INR > 1.5
    c. severe flu-like symptoms leading to discontinuation of therapy
    d. localized skin reaction from the injection (graded severe) leading to discontinuation of therapy
    e. coagulopathy/clinically significant prolongation of clotting time

Prior/Concurrent Clinical Study Experience

7. Participation in any clinical study in which they received an IMP within 4 months (or 5 times the half-life, whichever is longer) before Screening.

Diagnostic assessments

8. AST and ALT > 5 × ULN at Screening

    For individuals with any serum aminotransferase elevation > 2 × ULN, autoimmune hepatitis should be ruled out through the appropriate screening tests, which may include total IgG or gamma-globulin levels and/or serologic markers (i.e., antinuclear antibodies, anti-smooth-muscle antibodies at a titer of at least 1:40, anti-liver/kidney microsomal-1 antibodies, anti-liver cytosol antibody [anti-LC 1], or antisoluble liver/liver pancreas [anti-SLA/LP] antibodies).
9. ALP 2 × ULN at Screening.
10. Serum AFP value > 100 ng/mL at Screening.

    If AFP at screening is > ULN but < 100 ng/mL, the participant is still eligible if an appropriate hepatic imaging study reveals no lesions.
11. Positive screening for antimitochondrial antibodies (only required if primary biliary cholangitis is suspected).
12. Platelets < 100,000/mm^3 at Screening
13. INR > 1.6 × ULN at Screening

14. Positive screening for HBsAg, HCV antibodies, or HIV1 and 2 antibodies. If a participant has been tested in the past 3 months, medical record documentation of this testing can be used for eligibility.

    NOTE: In participants with previous treatment for hepatitis C with direct-acting HCV medication and seropositivity for HCV, or in participants with prior infection and spontaneous resolution, HCV RNA must be undetectable (at least 2 negative HCV RNA tests at least 12 weeks apart), and the HCV infection must have been resolved or cured > 3 years prior to enrollment.

5.3. Lifestyle Considerations

5.3.1. Meals and Dietary Restrictions

During this study, participants will be asked to:

- Abstain from heavy drinking throughout the study period, for example, no more than ≥ 21 units of alcohol per week in men and ≥ 14 units of alcohol per week in women (4 units per day for men and 3 units per day for women); where a "unit" of alcohol is equivalent to a 12-ounce beer, 4-ounce glass of wine, or 1 ounce shot of hard liquor as defined by the World Health Organization. Social drinking is allowed.

5.3.2. Activity

During this study, participants will be asked to:

- Abstain from strenuous exercise for 48 hours before each blood collection for clinical laboratory tests. Participants may participate in light recreational activities (e.g., walking at a pace < 3 miles per hour, shopping, watering plants) in that 48-hour time period.
- Plan to undertake no significant changes in exercise levels from the 48 hours prior to Day 1 until completion of the study, including F/U.

5.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomly assigned to the study intervention/entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the CONSORT publishing requirements, and to respond to queries from regulatory authorities. Minimal information includes, but are not limited to, demography, screen failure details, eligibility criteria, and any SAE.

Individuals who do not meet the criteria for participation in this trial (screen failure) may be rescreened once, at the discretion of the Investigator.

Rescreened participants will be assigned the same participant number as originally assigned preceded by the number “9.”

5.4.1. Screening Tests Required during the Rescreening Period

If a participant undergoes rescreening after being deemed a screen failure, the following screening assessments do not need to be repeated as part of the rescreening visit.

- Liver biopsy performed within 180 days (if the participant screen failed before a biopsy was performed, the liver biopsy must be collected as part of rescreening)
- PiZZ genotyping
- Viral serology (documented results within 3 months)
- Pulmonary X-ray (initial Screening pulmonary X-ray within 3 months of rescreening)
- FibroScan (initial Screening FibroScan within 30 days of the start of rescreening)
- MRE (initial Screening MRE within 30 days of the start of rescreening) (Cohort 1 & 2 only)
- Spirometry (initial Screening Spirometry within 30 days of the start of rescreening)
- DLCO (initial Screening DLCO within 30 days of the start of rescreening)
- Serum biomarkers (initial Screening serum biomarkers collected within 30 days of rescreening)

Any assessment not listed above must be performed again as part of the rescreening procedures.

6. STUDY INTERVENTION

Study intervention is defined as the investigational intervention, or placebo, intended to be administered to a study participant according to the study protocol.

6.1. Study Intervention Administered

Belcesiran is supplied as a solution of the drug substance (belcesiran sodium) in water for injection. The molecular mass of the sodium salt and free acid of the drug substance is 22286.2 Da and 21033.3 Da, respectively. The manufactured drug substance can be isolated only as a salt. As such, nonclinical and early clinical doses were expressed in terms of the sodium salt form of the drug substance. However, the United States Pharmacopeia 2013 “Salt Policy” specifies that when the active ingredient in a drug product is a salt, the strength should be expressed in terms of the active moiety rather than salt strength equivalent. For belcesiran, the sodium salt provides an additional 5.6% mass to the free acid. As such, the table below provides drug product concentrations and doses expressed in both the sodium salt and equivalent free acid (“active moiety”) weights.

Arm Name	Active		Placebo
Intervention name	belcesiran		0.9% saline for injection
Intervention type	Drug		Drug
Dose formulation	solution for injection		solution for injection
	Sodium salt	Free acid	
Drug product concentration	185 mg/mL	175 mg/mL	n/a
Route of administration	SC injection (thigh or abdomen)		SC injection (thigh or abdomen)
IMP or NIMP	IMP		n/a
Sourcing	Provided centrally by the Sponsor or designee		Provided locally by the trial site
Packaging and labeling	Belcesiran will be provided in vials. Each vial will be labeled as required per country.		n/a

Abbreviations: IMP: investigational medicinal product; n/a: not applicable; NIMP: non-investigational medicinal product; SC: subcutaneous.

6.1.1. Dose Level

Participants will receive belcesiran (or matching placebo) at a fixed dose of 210 mg in Cohorts 1 and 2 or a fixed dose of either 50 mg or 210 mg in Cohort 3. Cohorts 1 and 2 will be dosed monthly and Cohort 3 will be dosed monthly for the first 6 months and then quarterly thereafter. Participants in Cohort 1 will receive up to 13 doses, participants in Cohort 2 will receive up to 17 doses, and participants in Cohort 3 will receive 13 doses.

6.2. Preparation/Handling/Storage/Accountability

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit and storage at the site for all study intervention received, and any discrepancies are reported and approved by the Sponsor before the administration of the study intervention.

Only participants enrolled in the study may receive study intervention, and only authorized site staff may prepare or administer study intervention. All study intervention must be stored in a secure, environmentally controlled, and monitored (manual or automated) area per the labeled storage conditions with access limited to authorized site staff.

The authorized site staff are responsible for study intervention accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study interventions are provided in the Pharmacy Manual.

Belcesiran is to be stored at 2°C to 8°C (inclusive). Belcesiran should be allowed to warm to room temperature for approximately 1 hour (but no more than 4 hours) before administration.

Further guidance and information regarding study interventions are provided in the Pharmacy Manual.

6.3. Measures to Minimize Bias: Randomization and Blinding

The study is a randomized, placebo-controlled, double-blind study, where the Investigator and the study participants are blinded to IMP and placebo. All sites will have unblinded staff responsible for preparing IMP. A subset of the Sponsor's study team will be unblinded. Details of unblinding, including the list of unblinded Sponsor team members, will be captured in the Study Specific Blinding Plan.

All participants will be centrally assigned to randomized study intervention using an IVRS/IWRS. Before the study is initiated, directions for the IWRS will be provided to each site.

Investigators will remain blinded to each participant's assigned study intervention throughout the course of the study. In order to maintain this blind, the unblinded site staff will be responsible for the preparation and dispensation of all study intervention and will endeavor to ensure that there are no differences in the time taken to dispense the study intervention following randomization. Additionally, the unblinded site staff will affix a translucent label over the syringe(s) to mask the contents.

The unblinded study monitor and any auditors present in the event of a Quality Assurance or a competent authority audit will be allowed access to unblinded study intervention records at the site(s) to verify that randomization/dispensing has been done accurately and per protocol and the Pharmacy Manual.

In case of an emergency, the Investigator has the sole responsibility for determining if unblinding of a participant's treatment assignment is warranted. Participant safety must always be the first consideration in making such a determination. If a participant's treatment assignment is unblinded, the Medical Monitor must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation.

Potentially unblinding study results, such as the serum AAT protein concentrations, will not be shared with blinded study participants and site personnel during the treatment period.

6.4. Study Intervention Compliance

Study intervention will be administered at the investigational site or home (after the first dose) if the participant is unable to come to the study site. The planned dose, actual administered dose, and injection date and time will be recorded in the source documentation.

6.5. Concomitant Therapy

Any medication or vaccine (including OTC or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving within 30 days prior to Screening or receives during the study treatment period must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

6.5.1. Augmentation Therapy

Study participants who are receiving augmentation therapy prior to study entry should continue their augmentation therapy on a regular basis for the duration of the study.

For study participants who are not receiving augmentation therapy prior to study entry, augmentation therapy is available in case of a clinically significant worsening in lung function at the discretion of the investigator during the entire trial. Only doctors with augmentation therapy experience should make the decision to initiate the treatment. The date, time, and dosage regimen of intravenous purified AAT administration must be recorded.

6.5.2. Vaccinations

Where possible, to allow discernment of AEs related to the administration of a vaccine from those related to administration of study intervention, vaccines should not be administered in the 7 days prior to or following scheduled administration of study intervention.

6.6. Dose Modification

No dose modifications for individual participants will be allowed.

6.7. Intervention after the End of the Study

Upon completion of the study, Investigators will discuss care options with participants.

7. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1. Discontinuation of Study Intervention

Treatment of a participant may be discontinued at any time during the trial at the discretion of the investigator for safety, behavioral, compliance or administrative reasons. Efforts must be made to have participants, who discontinue trial product, continue in the trial to evaluate safety and serum AAT protein concentrations. Participants must be educated about the continued scientific importance of their data, even if they discontinue trial product. Data to be collected at the time of intervention discontinuation and follow-up and for any further evaluations that need to be completed are outlined in the SoA (Section 1.3). Only participants who withdraw consent will be considered as withdrawn from the trial.

7.1.1. Stopping Rules for Individual Participants

Discontinuation of treatment can be decided by either the Investigator or the participant. Participants who discontinue trial product should continue with the scheduled visits and assessments. If the participant does not wish to attend the scheduled clinic visits, efforts should be made to have visits converted to phone contacts. However, all efforts should be made to have the participant attend the visits at week 24, 48 and 96, as applicable.

The trial product must be discontinued, if any of the following applies for the participant:

1. Decline in Pulmonary Function (see Section 7.1.1.1)

2. Abnormal liver blood parameters indicating DILI (see Section 7.1.1.2)

3. Pregnancy/ Intention of becoming pregnant

4. Simultaneous use of an approved or non-approved investigational medicinal product in another clinical trial

5. Safety concerns as judged by the Investigator

Participants meeting any of the above treatment discontinuation criteria are allowed to resume trial product, if the criterion is no longer met.

For Germany: please also see country-specific requirements in Section 10.5.

7.1.1.1. Decline in Pulmonary Function

For participants not on augmentation therapy, the occurrence of the events detailed as A and B below will result in temporary discontinuation of study intervention in the affected participant. These events will necessitate review and adjudication by the DSMC. If, in the opinion of the DSMC, the decline in pulmonary function (as defined by events A or B, below) is likely related to something other than the study drug, e.g., a common cold, dosing in that participant may continue, otherwise, dosing of the participant can be permanently discontinued at the discretion of the Investigator.

A. **FEV_1:** At least 2 pulmonary function tests showing relative decrease from baseline of ≥ 15% AND an absolute decrease from baseline of > 200 mL in FEV_1 following administration of bronchodilators over a period of at least 3 months.

B. **DLCO:** At least 2 pulmonary function tests showing a relative decrease from baseline of ≥ 15% in Hb-corrected DLCO AND an absolute decrease from baseline of > 6.5 mL/min/mmHg in Hb-corrected DLCO, following administration of bronchodilators over a period of at least 3 months.

7.1.1.2. Drug-Induced Liver Injury Monitoring

For Participants with Normal Liver Biochemistries at Baseline

Study intervention will be discontinued when a participant meets one of the conditions outlined below (from Guidance for Industry-Drug Induced Liver Injury: Premarketing Clinical Evaluation [FDA, 2009]) or if the Investigator believes that it is in the best interest of the participant. Safety laboratories will be monitored by a central laboratory in real time with potential drug-induced liver injury (DILI) alerts sent to the Investigator.

- ALT or AST > 8 × ULN
- ALT or AST > 5 × ULN for more than 2 weeks
- ALT or AST > 3 × ULN and (total bilirubin >2 × ULN or INR >1.5)
- ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (> 5%)

Participants should be monitored at each study visit and instructed to call the Investigator for new or worsening symptoms of clinical hepatitis. Both symptoms and calls should be documented in source notes.

NOTE: Any elevation in an LFT leading to discontinuation of study intervention must be at least possibly related to the investigational drug, and other potential causes (e.g., strenuous exercise, use of concomitant medication) must have been excluded.

Alternative DILI Monitoring Algorithm for Participants with Elevated Transaminases at Baseline

The following alternative DILI monitoring algorithm should be used in participants with elevated transaminases above ULN at baseline.

- **Close Monitoring**: If participants develop elevations of AST or ALT > 2 times the screening value or total bilirubin > 1.5 times the screening value while on the study, testing should be repeated within 48 to 72 hours. Persistent elevations should be followed by repeat testing and physical examinations 2 to 3 times per week with or without drug discontinuation.
- **Study Intervention Discontinuation**: Study intervention will be discontinued if a participant meets one of the following abnormal liver function conditions:
  - ALT or AST > 5 × the screening value in participants with a screening value < 2 × ULN

- ALT or AST > 3 × the screening value in participants with a screening value ≥ 2 × ULN but < 5 × ULN
- ALT or AST increase > 2 × the screening value accompanied by a concomitant total bilirubin increase to > 2 × the screening value OR an INR increase of > 0.2
- Elevations of liver enzymes accompanied by symptoms consistent with hepatic injury (e.g., fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia [> 5%])

Assessments in Case of Increased Levels of Liver Blood Parameters

For all such events listed above repeated testing must occur within 48 to 72 hours and work up for competing etiologies must be performed including:

- Complete liver profile including ALT, AST, alkaline phosphatase, conjugated bilirubin, total bilirubin and INR. Hereafter repeat testing should be done 2 to 3 times weekly. If close monitoring is not possible the trial product should be discontinued. The frequency of repeat testing can decrease to once a week or less if abnormalities stabilize and the participant is asymptomatic.
- A detailed history of symptoms and prior or concurrent diseases.
- History of concomitant drug use (including non-prescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
- Ruling out acute viral hepatitis (including hepatitis A, B, C and E); autoimmune or alcoholic hepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease.
- Obtaining a history of exposure to environmental chemical agents.

Trial product can be restarted if an alternative etiology is definitively identified, and liver blood parameters have returned to pre-event levels.

7.1.2. Study Stopping Rules

All further enrollment and dosing in the study will be stopped if any of the stopping rules defined in the DSMC charter are met and the DSMC recommends stopping the study.

The Sponsor will promptly notify all sites if stopping rules are triggered.

NOTE: In the event that a study stopping rule is triggered, all ongoing participants will continue to be followed for safety assessments as indicated in the SoA (Section 1.3).

7.2. Participant Discontinuation/Withdrawal from the Study

A participant may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, compliance, or administrative reasons (i.e., ET). This is expected to be uncommon.

At the time of discontinuing from the study, if possible, an early termination visit should be conducted, as shown in the SoA (Section 1.3). Data to be collected at the time of study

discontinuation, during follow-up, and for any further evaluations that need to be completed, provided the participant did not withdraw consent, are outlined in the SoA.

If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a participant withdraws from the study, he/she may request the destruction of any samples taken and not tested, and the Investigator must document this in the site study records.

The reason for participant discontinuation or withdrawal from the study will be recorded on the CRF.

Participants who sign the ICF, are randomized and subsequently withdraw or are withdrawn or discontinued from the study, may be replaced to meet the minimum requirements of sample size described in the study design (Section 4.1).

7.3. Lost to Follow up

A participant will be considered lost to follow-up if he or she fails to return for 3 consecutive visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the Investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls, and if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8. STUDY ASSESSMENTS AND PROCEDURES

The timing of study procedures is summarized in the SoAs (Section 1.3). Protocol waivers or exemptions are not allowed.

Immediate safety concerns should be discussed with the Medical Monitor upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.

Adherence to the study design requirements, including those specified in the SoAs (Section 1.3), is essential and required for study conduct.

Informed consent must be obtained before any trial related activity (Section 10.1.3)

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

The maximum amount of blood drawn from each participant at any visit (including PK, PD, and safety laboratory testing) will not exceed:

- 132 mL

The total amount of blood collected (including PK, PD, and safety laboratory testing) will not exceed:

- 480 mL in 28 days or
- 550 mL in 8 weeks

8.1. Efficacy Assessments

- The following sections describe the assessments and procedures while their timing is specified in the SoAs (Section 1.3).
- Phone visits may be replaced by a site visit at the discretion of the Investigator.
- Repeat samples may be taken for technical issues and additional samples or assessments may be taken for safety reasons. Please refer to Appendix 2, Section 10.2 for further details on laboratory samples.
- Laboratory tests may be analyzed on either an on-going basis, at end-of-treatment or at end-of-trial, depending on the test.

8.1.1. Serum Z-AAT Protein Concentration

Venous blood samples of approximately 16 mL will be collected for the measurement of serum Z-AAT protein concentrations at time points specified in the SoA (Section 1.3). Instructions for the collection and handling of samples will be provided in the Lab Manual.

8.1.2. Assessment of Fibrosis and Hepatic Z-AAT Content via Percutaneous Liver Biopsy

Percutaneous non-targeted liver biopsies will be performed to assess eligibility to the study and quantify the change in hepatic Z-AAT protein content and histology.

Liver biopsy will be performed according to the study-specific Liver Biopsy Manual. The first biopsy will be performed at Screening once all other eligibility criteria are met, and the participant is qualified for enrollment. The second liver biopsy will be at either 24 weeks (Cohort 1 and 3) or 48 weeks (Cohort 2) after administration of the first dose of the study intervention. Finally, there will be an optional liver biopsy at week 96.

The participant's vital signs will be monitored during the biopsy procedure per local standard of care. As post-biopsy care, the participant will be observed per local standards of care for any signs or symptoms of bleeding, hematoma, and pain.

Liver tissue will be processed on-site, in accordance with the study-specific Liver Biopsy Manual (e.g., snap frozen or formalin-fixed) and sent to a central laboratory for pathology. A clinical report will be generated and sent to the Principal Investigator. In addition, the histology assessment might be supplemented by digital pathology evaluation.

8.1.2.1. Liver Histology: Ishak Fibrosis Staging

The Ishak staging system for liver fibrosis is a 1995 update to the algorithm initially developed by De Groote et al. (Ishak et al., 1995; De Groote et al., 1968). Ishak scores range from 0 (no fibrosis) to 6 (cirrhosis) as summarized in Table 55.

Table 55: Ishak Fibrosis Staging

Stage	Description
0	No fibrosis
1	Fibrous expansion of some portal areas, with or without short fibrous septa
2	Fibrous expansion of most portal areas, with or without short fibrous septa
3	Fibrous expansion of most portal areas with occasional portal-to-portal bridging
4	Fibrous expansion of portal areas with marked bridging (portal to portal as well as portal to central)
5	Marked bridging (portal–portal and/or portal–central) with occasional nodules (incomplete cirrhosis)
6	Cirrhosis, probable or definite

8.1.2.2. Liver Histology: METAVIR Scoring System

In 1996, The METAVIR Cooperative Study Group developed an algorithm to generate a single score of necroinflammatory liver activity based on basic histological features (Bedossa & Poynard, 1996). The resultant score is composed of a 2-letter and 2-number coding system that describes activity (A) and fibrosis (F), as displayed in Table 66.

Table 66 METAVIR Scoring System

Fibrosis score:	Activity score:
F0 = no fibrosis	A0 = no activity
F0 – F1	A0 – A1
F1 = portal fibrosis without septa	A1 = mild activity
F1 – F2	A1 – A2
F2 = portal fibrosis with few septa	A2 = moderate activity
F3 = numerous septa without cirrhosis	A2 – A3
F3 – F4	A3 = severe activity
F4 = cirrhosis	

8.1.2.3. Liver Histology: Additional Parameters

In relation to the liver histology evaluation, additional histology parameters, such as presence of liver globules, are assessed. Further details can be found in the Liver Biopsy Manual.

8.1.2.4. Hepatic Z-AAT Content

Measurements of Z-AAT in liver tissues will be performed. Instructions for the collection and handling of samples will be provided in the Lab Manual.

8.1.3. Noninvasive Measures of Liver Fibrosis

Noninvasive measures of liver fibrosis will be measured at time points specified in the SoA (Section 1.3). The blood biomarkers to be assessed are detailed in Sections 8.1.3.1 through 8.1.3.5. Full details regarding the collection, preparation, and handling of samples will be provided in the laboratory manual. Note that the list of blood biomarkers detailed is not comprehensive, and biomarkers may be added or deleted as deemed appropriate.

8.1.3.1. Biochemical Fibrosis Index

The FibroSure fibrosis index was first developed as a non-invasive alternative to liver biopsy for staging of fibrosis in patients with chronic hepatitis C (Imbert-Bismut et al., 2001). Multiple studies have since demonstrated that the diagnostic value of the index is comparable to that of liver biopsy in assessing fibrosis across a range of liver diseases (Ratziu et al., 2006; Zeremski et al., 2014; Lurie et al., 2015). The fibrosis index utilizes 10 biochemical markers (α-2 macroglobulin, haptoglobin, GGT, ALT, AST, total bilirubin, apolipoprotein A1, total cholesterol, triglycerides, and serum glucose) plus age and gender to provide fibrosis staging.

8.1.3.2. Aspartate Aminotransferase to Platelet Ratio Index

The APRI is calculated using the AST level (corrected for the ULN) and platelet count:

$$APRI = \frac{\frac{AST}{AST\ ULN}}{platelet\ count} * 100$$

A meta-analysis of 40 studies found that an APRI cutoff of ≥ 0.7 had an estimated sensitivity of 77% and specificity of 72% for detection of significant hepatic fibrosis (≥ F2 by METAVIR; Lin et al., 2011).

8.1.3.3. FIB-4

The FIB-4 is an index based on age, AST, ALT, and platelet count:

$$FIB\text{-}4 = \frac{Age\ x\ AST}{Platelet\ count * \sqrt{ALT}}$$

In patients with HCV infection, a FIB-4 index < 1.45 was shown to have a negative predictive value of 94.7% to exclude severe fibrosis with a sensitivity of 74.3%. A FIB-4 index > 3.25 had a positive predictive value to confirm the existence of significant fibrosis (F3-F4) of 82.1% with a specificity of 98.2%. The FIB-4 was found to correlate strongly ($P < 0.001$) with FibroTest results (Vallet-Pichard et al., 2007).

8.1.3.4. Enhanced Liver Fibrosis Score

The ELF score is an extracellular matrix marker set consisting of tissue inhibitor of metalloproteinases 1 (TIMP-1), amino-terminal propeptide of type III procollagen (PIIINP), and hyaluronic acid (HA). Lichtinghagen et al. identified three cut-off values for the ELF Score in predicting fibrosis: 7.7 for a high sensitivity exclusion of fibrosis, 9.8 for a high specificity identification of fibrosis (sensitivity 69%, specificity 98% for moderate fibrosis), and 11.3 to discriminate cirrhosis (sensitivity 83%, specificity 97%), but noted that age and gender must be taken into account (Lichtinghagen et al., 2013).

8.1.3.5. Other Markers of Fibrosis or Liver Inflammation

Other blood biomarkers to be measured may include:

- Pro-C3, tenascin, CK-18, M30, and M65

8.1.4. Assessment of Liver Stiffness

Assessment of liver stiffness via MRE (only Cohorts 1 and 2) and/or FibroScan will be measured at time points specified in the SoAs (Section 1.3).

8.1.4.1. FibroScan

Liver stiffness measurement (LSM) is evaluated by measuring the velocity of a vibration wave generated on the skin by the FibroScan (Echosens, Paris, France) device at sites where the equipment is available. The LSE is the median of the successful stiffness measurements and has a range of 2.5 kPa (lowest stiffness) to 75 kPa (highest stiffness). At sites where FibroScan® equipment with Controlled Attenuation Parameter (CAP) option is available, measurements of liver steatosis must also be performed.

The liver stiffness and CAP measurements must be performed in a fasting state (fasting for at least 3 hours) and according to site standard procedures.

8.1.4.2. Magnetic Resonance Elastography (Cohorts 1 and 2 only)

MRE is a non-invasive technique that uses propagating mechanical shear waves ranging from 20 to 200 Hz, to determine liver stiffness. The MRE hardware typically consists of an active acoustic driver coupled with a disc-shaped non-metallic passive driver that is placed against the right anterior chest wall overlying the liver. Usually, a continuous acoustic vibration of 60 Hz is transmitted into the abdomen via the passive driver. Shear waves propagate more rapidly through stiffer liver tissue (longer wavelength) indication of liver fibrosis than the softer healthy liver tissue (shorter wavelength). The imaging process is accomplished in one or more breath-holds, and the images are processed with MRE specialized software to generate quantitative cross-sectional elastograms depicting the liver stiffness (Venkatesh et al., 2013).

The technique is useful for differentiating patients with clinically important hepatic fibrosis (F1 to F4) from those without (Yin et al., 2007; Kim et al., 2016).

Central overread of MRE will be performed to ensure consistency of findings across sites. Full details regarding the conduct and central overread of the MRE will be provided in a separate manual.

All participants in Cohorts 1 and 2 should have the MRE assessment completed unless access to the equipment and technology is not available. The MRE hardware and software must be locally approved for diagnostic medical imaging. If the site does not have MRE hardware and software that is locally approved for diagnostic medical imaging, MRE assessments will be optional.

8.1.5. Omics Analyses

In order for further characterize and quantify the effect of belcesiran on biological molecules, such as proteins (proteomics) or RNA molecules (transcriptomics), omics analyses may be performed using validated methods such as the Somalogic Platform.

8.1.6. St. George's Respiratory Questionnaire

The SGRQ is designed to measure health impairment in patients with asthma and COPD and is comprehensive, covering a range of aspects of COPD (Cazzola et al, 2008). This instrument has 3 domains: symptoms; activity; and impacts. A total score is also calculated. It was designed for supervised self-administration.

This assessment will be conducted as indicated in the SoA (Section 1.3).

8.1.7. Chronic Liver Disease Questionnaire

The Chronic Liver Disease Questionnaire (CLDQ) was developed as an evaluative instrument to measure longitudinal change in health status within individuals with chronic liver disease (Chawla et al, 2016). In addition to measuring both physical and mental health, the instrument was designed to be a disease-specific tool for assessing areas of function important to patients with chronic liver disease.

This assessment will be conducted as indicated in the SoA (Section 1.3).

8.1.8. Covid-19 Testing

No routine COVID-19 testing is planned during the trial. COVID-19 testing may be performed at any time during the study as required by local requirements or deemed necessary by the investigator or by Sponsor. Laboratory results will not be transferred to the study database, but COVID-19 infection should be registered as concomitant illness or AEs.

8.2. Safety and Other Assessments

Planned time points for all safety assessments are provided in the SoA (Section 1.3). Unless otherwise specified, a ± 10-minute window around each time point is allowed if multiple assessments are due at the same time. In such cases, the order of priority should be ECG, vital signs, PK, and then other assessments, with the PK sampling preferably at the nominal time point. Planned time points for all safety assessments are provided in the SoA (Section 1.3).

Investigators are able to schedule visits if needed and to convert phone visits to site visits if needed.

8.2.1. Screening Assessments

A chest x-ray must be performed during Screening to establish a baseline for comparison. If the participant had been imaged in a stable condition within the prior 4 weeks, that x-ray may be used if the images are available.

In addition to diagnostic testing performed throughout the treatment period (and F/U, as applicable), the following laboratory testing will be performed during the Screening Period:

- HbA1c
- Viral serology: HBsAg, HCV antibodies, or HIV1 and 2 antibodies. If a participant has been tested in the past 3 months, medical record documentation of this testing can be used for screening.
- Urine drug screen (to include at a minimum: amphetamines, barbiturates, cocaine, opiates, and benzodiazepines)
- Amount of cotinine in urine to assess smoking status at Screening.

Additionally, as indicated:

- Follicle stimulating hormone (FSH), to confirm postmenopausal status
- Suspected primary biliary cholangitis should be ruled out via antimitochondrial antibody testing
- Suspected autoimmune hepatitis should be ruled out through the appropriate screening tests, which may include total IgG or gamma-globulin levels and/or serologic markers (antinuclear antibodies, anti-smooth-muscle antibodies at a titer of at least 1:40, anti-liver/kidney microsomal-1 antibodies, anti-liver cytosol antibody [anti-LC 1], or anti-soluble liver/liver pancreas [anti-SLA/LP] antibodies).

8.2.2. Physical Examinations

A complete physical examination will be required at visits as indicated in the SoA (Section 1.3) and will include, at a minimum, assessments of the eyes, ears, nose, throat, thyroid/neck, lymph nodes, as well as cardiovascular, respiratory, gastrointestinal, musculoskeletal, dermatological, and neurological systems. Height (in cm, only at Screening), weight (in kg), and body mass index will also be measured and recorded. Height and weight should be measured without shoes.

In lieu of a complete physical examination, a focused physical examination will be performed at other visits indicated in the SoA (Section 1.3). It will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

Investigators should note any pre-existing clinical signs related to previous illnesses.

For at-home visits (whenever applicable), the home-health nurse will provide in-person support to the Investigator in assessing the participant.

Study intervention injection sites should be inspected at each visit.

8.2.3. Vital Sign Measurements

Temperature (per local standard of care), pulse rate, respiratory rate, and blood pressure will be assessed.

Blood pressure and pulse measurements will be assessed in the sitting position with a completely automated device. Manual techniques will be used only if an automated device is not available. Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., television, cell phones).

Temperature will be obtained in degrees Celsius. The pulse rate should be counted for a full minute and recorded in beats per minute. Respirations should be counted for a full minute and recorded in breaths per minute.

8.2.4. Electrocardiograms

Standard 12-lead ECGs will be performed after the participant has rested comfortably for 5 minutes. Where feasible, ECGs will be obtained using a machine that automatically calculates the heart rate and ventricular rate and measures RR, PR, QRS, QT, and corrected QT interval (QTcF, Fridericia correction). The Investigator or their qualified designee is responsible for reviewing the ECG(s) to assess whether the results are within normal limits and to determine the clinical significance of the results.

8.2.5. Pulmonary Function Tests

8.2.5.1. Spirometry

Definitions:

- FVC is the maximal volume of air exhaled with maximally forced effort from a maximal inspiration, i.e., VC performed with a maximally forced expiratory effort, expressed in liters.

- FEV_1 is the maximal volume of air exhaled in the first second of a forced expiration from a position of full inspiration, expressed in liters.

Procedure:

- Spirometry will be performed per the ATS/ERS standards (Miller et al., 2005). A nose clip should be used for spirometry testing. Spirometry will be performed postadministration of bronchodilators. At screening, spirometry will also be performed pre-bronchodilator administration.

There are 3 distinct phases to the FVC maneuver:

- maximal inspiration
- a blast of exhalation; and
- continued complete exhalation to the end of the test

The participant should inhale rapidly and completely. The breathing tube should be inserted into the participant's mouth (if this has not already been done), making sure the lips are sealed around the mouthpiece and that the tongue does not occlude it. Then the FVC maneuver should begin immediately.

It is important that the participant takes a complete inhalation before beginning the maneuver. The participant should be prompted to "blast," not just "blow," the air from their lungs, and then be encouraged to exhale fully.

An adequate test requires at least 3 acceptable FVC maneuvers. If acceptability criteria are not met in 3 attempts, additional trials should be attempted, up to not more than 8 total attempts.

Full details regarding the conduct of and the criteria for acceptability of spirometry testing will be provided in a separate manual.

8.2.5.2. Diffusion Capacity of the Lungs for Carbon Monoxide

Definition:

- DLCO is a measure of the quantity of CO transferred per minute from alveolar gas to red blood cells (specifically hemoglobin) in pulmonary capillaries. DLCO is expressed as mL/min/mmHg, that is, the milliliters of CO transferred per minute for each millimeter of mercury in pressure difference across the total available functioning lung gas exchange surface.

Procedure:

- DLCO will be performed per the ATS/ERS standards (Graham et al., 2017). The same calibrated equipment must be utilized throughout the study.
- DLCO will be performed prior to spirometry testing.
- Participants should be seated throughout the procedure and should be allowed several minutes of quiet breathing prior to testing to become accustomed to the mouthpiece and nose clip. A minimum of 2 acceptable maneuvers should be completed, with values within 2.00 mL/min/mmHg (0.67 mmol/min/kPa) of each other. Participants should be given a 4-minute window between maneuvers to allow for adequate

elimination of the test gas from the lungs. Not more than 5 maneuvers should be attempted.

Full details regarding the conduct of and the criteria for acceptability of DLCO will be provided in a separate manual.

8.2.6. Other Pulmonary Information

As indicated in the SoA (Section 1.3), at each timepoint for which PFTs are performed, the following information should be collected and noted in the eCRF:

- Number and severity of pulmonary exacerbations (characterized by dyspnea and/or cough and sputum that worsen over ≤14 days) since the last visit;
  - Mild exacerbation: Worsening of symptoms that can be self-managed and resolved without systemic corticosteroids or antibiotics.
  - Moderate exacerbation: Increase in symptoms that requires treatment with antibiotics and/or corticosteroids
  - Severe exacerbation: Need for hospitalization
- Smoking status, including number of cigarettes (or equivalent) per day
- Use of AAT replacement therapy (yes/no) since the last visit
- Modified Medical Research Council dyspnea score (adapted from Fletcher et al., 1959)
  - Grade 0: The patient only gets breathless with strenuous exercise
  - Grade 1: The patient gets short of breath when hurrying on level ground or walking up a slight hill
  - Grade 2: On level ground, the patient walks slower than people of the same age because of breathlessness, or have to stop for breath when walking at their own pace
  - Grade 3: The patient stops for breath after walking about 100 yards or after a few minutes on level ground
  - Grade 4: The patient is too breathless to leave the house or is breathlessness when dressing
- Resting peripheral oxygen saturation

8.2.7. Clinical Safety Laboratory Assessments

See Section 10.2 for the list of clinical laboratory tests to be performed and the SoA (Section 1.3) for the timing and frequency. Participants should be fasting for a minimum of 8 hours prior to the collection of blood samples as indicated in the SoA.

The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. The laboratory reports must be filed with the source documents.

All laboratory tests with values considered clinically significantly abnormal during participation in the study should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or Medical Monitor.

- If such values do not return to normal/baseline within a time judged reasonable by the Investigator, the etiology should be identified, and the Sponsor notified.
- If laboratory values from non-protocol-specified laboratory assessments performed at the institution's local laboratory require a change in participant management or are considered clinically significant by the Investigator (e.g., SAE or AE) they must be recorded in the eCRF.

8.3. Adverse Events and Serious Adverse Events

The definitions of AEs and SAEs are located in Section 10.3.

The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain accountable for following up AEs that are serious, considered related to the study intervention or study procedures, or that caused the participant to discontinue the study intervention (see Section 7.1).

8.3.1. Time Period and Frequency for Event Assessment

All AEs/SAEs will be collected from the signing of the ICF until the last day of study participation, including F/U.

Any medical condition that is present at the time that the participant is screened will be considered as baseline medical history and not reported as an AE. However, if the study participant's condition deteriorates after having received the first dose of Investigational Product during the study, it will be recorded as a TEAE.

Investigators are not obligated to actively seek AE or SAE after the conclusion of the study participation. However, if the Investigator learns of any SAE, including death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the Investigator must notify the Sponsor promptly.

8.3.2. Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting SAE reports are provided in Section 10.3.

AE or SAEs will be reported by the participant or observed by the study staff. Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences, for example, "Have you noticed anything different since you started the study?". The occurrence of an AE or SAE may also be detected upon review by a study monitor.

8.3.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs and non-serious AESI; as defined in

Section 10.3.3) will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is given in Section 10.3.

8.3.4. Serious Adverse Event Reporting

All SAEs will be recorded and reported to the Sponsor or designee immediately; under no circumstance should this exceed 24 hours, as indicated in Section 10.3.5. The Investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

Prompt notification by the Investigator to the Sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of the study intervention under clinical investigation are met.

The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC, and Investigators.

Investigator safety reports will be prepared for SUSAR according to local regulatory requirements and Sponsor or Sponsor's designee's policy and will be forwarded to Investigators as necessary.

8.3.5. Reporting of Pregnancy

Details of all pregnancies in female participants and female partners of male participants will be collected after the start of study intervention and until 24 weeks after the last dose.

If a pregnancy is reported, the Investigator should inform the Sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Section 10.4.4.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE. Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

The participant or pregnant partner will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the participant or pregnant partner and the neonate, and the information will be forwarded to the Sponsor.

Any post-study pregnancy-related SAE considered reasonably related to the study intervention by the Investigator will be reported to the Sponsor as described in Section 8.3.4. While the Investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

8.3.6. Adverse Events of Special Interest

AEs of special interest (AESI; as defined in Section 10.3.3), will be followed until resolution or stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3).

8.4. Treatment of Overdose

For this study, any dose of study intervention greater than the protocol-specified dose will be considered an overdose.

In the event of an overdose, the Investigator should:

1. Immediately contact the Medical Monitor.
2. Closely monitor the participant for any AE/SAE and laboratory abnormalities for at least 14 days.
3. Obtain a plasma sample for PK analysis within 14 days from the date of the last dose of study intervention if requested by the Medical Monitor (determined on a case-by-case basis).
4. Document the quantity of the total dose as well as the number of injections over time.

Decisions regarding dose interruptions will be made by the Investigator in consultation with the Medical Monitor based on the clinical evaluation of the participant.

8.5. Pharmacokinetics

Blood samples of approximately 2.0 mL will be collected for the measurement of plasma concentrations of belcesiran, as specified in the SoA (Section 1.3).

Instructions for the collection and handling of biological samples will be provided by in the laboratory manual. The actual date and time (24-hour clock time) of each sample will be recorded.

Samples collected for analyses of belcesiran concentration may also be used to evaluate safety or efficacy aspects related to concerns arising during or after the study.

Pharmacokinetic parameters to be determined include apparent clearance (CL/F) and apparent volume of distribution (Vz/F), AUC, C_{max}, T_{max}, and $t_{1/2}$, as data permit to determine the PK profile of belcesiran.

Drug concentration information that would unblind the study will not be reported to investigative sites or blinded personnel.

8.5.1. Metabolite Identification

Plasma metabolite identification studies may be performed by nuclear magnetic resonance, mass spectrometry, liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry, Fourier transform mass spectrometry, and/or equivalent methods.

8.6. Pharmacodynamics

Pharmacodynamic parameters, other than serum Z-AAT protein concentrations (as described in Section 8.1.1), are not evaluated in this study.

8.7. Genetics

Genetics are not evaluated in this study. Genotyping will be performed locally at screening if historical data are not available for determination of study eligibility for the participants.

8.8. Immunogenicity Assessments

Antibodies to belcesiran will be evaluated in serum samples collected from all participants, according to the SoA (Section 1.3). Additionally, serum samples should also be collected at the final visit from participants who discontinued study intervention or were withdrawn from the study. These samples will be tested by the Sponsor or Sponsor's designee.

Samples for the detection and characterization of antibodies to belcesiran will be stored until a validated assay method is available.

8.9. Medical Resource Utilization and Health Economics

Medical resource utilization and health economics will not be evaluated in this study.

9. STATISTICAL METHODS

In general, summary statistics (n, mean, standard deviation, median, minimum and maximum values for continuous variables, and number and percentage of participants in each category for categorical variables) will be provided by treatment group and cohort for all variables.

Baseline value is defined as the last non-missing assessment prior to the first dose of study drug, except for serum AAT where baseline is defined as the mean from all predose AAT measurements.

Source data for the summary tables will be presented as by-subject data listings.

All statistical tests will be 1-sided with a type I error rate of 5%, unless otherwise specified.

Complete details of the statistical analysis will be outlined in the SAP and finalized before the interim analysis database lock.

9.1. Randomization and Sample Size

The study will include 3 cohorts. In Cohorts 1 and 2 there will be up to 8 participants in each cohort (randomization scheme ratio of 3:1 active:placebo). The sample size for Cohort 1 and Cohort 2 is based on clinical consideration rather than statistical power calculation.

The primary endpoint for Cohort 3 of this study is the percentage change from baseline in serum and liver Z-AAT protein concentration at week 24. Assuming the percentage change from baseline is 80%, 60% and 10% in the active high dose group, active low dose group and placebo group respectively, and the standard deviation is 0.5 for all three groups, a sample size of 10 participants per treatment group will provide at least 80% power to detect a statistically significant difference between active high dose group and placebo, and between active low dose group and placebo, at an overall 1-sided significance level of 0.05.

The F1, F2, F3, and F4 fibrosis types will be used as randomization stratification factors for all 3 cohorts.

9.2. Study Populations

For purposes of analysis, the following populations are defined:

- **Enrolled:** All participants who sign the informed consent
- **Safety:** All participants randomly assigned to study intervention and who received at least 1 dose of belcesiran/placebo. Participants will be analyzed according to initial dose received.
- **Pharmacokinetic:** All participants randomly assigned to study intervention and who received at least 1 dose of belcesiran and have at least 1 postdose PK assessment
- **Pharmacodynamic**: All participants randomly assigned to study intervention and who received at least 1 dose of belcesiran/placebo and have at least 1 postdose PD assessment
- **Evaluable**: All participants randomly assigned to study intervention and who received at least 50% of planned doses of belcesiran/placebo

Additional subpopulations may be included for sensitivity analysis. These will be detailed in the SAP.

9.3. Statistical Analyses

9.3.1. Analyses of Primary Endpoints

9.3.1.1. Safety Analysis

All safety analyses will be performed on the Safety Population. Adverse events will be defined as TEAEs if they have a start date on or after the administration of study drug during the treatment period, or if they occur prior to the administration of study drug and worsen in severity/grade or relationship to the study intervention after the administration of study intervention during the treatment period. Frequency and percentages will be used to summarize TEAEs, SAEs, and AEs of special interest by relationship. Laboratory values will be reported by grade and summarized, if applicable.

Change from baseline and observed values will be summarized where applicable. Laboratory analytes will be presented by a shift from baseline and graphically represented in mean values over time where applicable. Vital signs, ECG, and PFTs (i.e., FEV_1, FVC, FEV_1/FVC, and DLCO) will be summarized as continuous parameters.

9.3.1.2. Analysis of Serum and Liver Z-AAT Concentrations

The primary endpoint for Cohort 3 of this study is the percentage change from baseline in serum and liver Z-AAT protein concentration at week 24.

The primary endpoint for Cohort 3 will be analyzed by using a linear log-normal regression model. Statistical testing for the primary endpoint of Cohort 3 will be carried out using the Hocheberg testing procedure for the following comparisons: (active high dose vs. placebo) and (active low dose vs. placebo). The linear log-normal regression model will include treatment, baseline value (log transformed) and randomization stratification factor of fibrosis types (F1, F2, F3, and F4) as covariates.

9.3.2. Analyses of Secondary Endpoints

9.3.2.1. Pharmacokinetic Analyses

Pharmacokinetic analyses will be performed on all participants who received at least 1 dose of belcesiran and have sufficient data for at least one evaluable postdose PK assessment. Actual sampling times will be used for all parameter estimations.

Plasma concentrations and PK parameters will be listed and summarized by group and by time point with descriptive statistics. The arithmetic mean, SD, CV%, geometric mean, geometric CV%, minimum, median, and maximum will be presented.

Additional PK parameters may be estimated if deemed appropriate. Details will be provided in the PK Analysis Plan. Analysis of PK parameters may be reported in a supplement to the CSR.

9.3.2.2. Analyses of Fibrosis, Steatosis, and Inflammation

Changes from Baseline in liver histology including METAVIR and Ishak scores will be analyzed by descriptive statistical methods. METAVIR and Ishak scores will summarized and, if applicable, analyzed using modeling techniques, which will be described in the SAP.

9.3.3. Exploratory Analyses

Exploratory analyses will be conducted as deemed appropriate and will be detailed in the SAP.

Descriptive summaries will be provided for the following exploratory endpoints: FibroScan® score, ELF score, and CK-8. Values, changes, and percent change for each parameter will be analyzed, when applicable.

Further supporting analyses such as correlation analyses between biomarkers and/or liver histology parameters might be performed to better understand the effect of belcesiran, if applicable.

9.4. Interim Analyses

There are 3 interim analyses (IAs) planned for this study. The first interim analysis (IA1) will be conducted when all participants in Cohort 1 have completed 24 weeks of treatment. The second interim analysis (IA2) will be performed when all participants in Cohort 2 have completed 48 weeks of treatment. The third interim analysis (IA3) will be performed when all the participants in Cohort 3 have completed 24 weeks of treatment. As part of the IAs, all study site personnel will remain blinded, but the Sponsor will be unblinded. Details of unblinding including the list of unblinded Sponsor team members, will be described in the Study Specific Unblinding Plan. Additional interim analyses may be performed as needed.

The unblinded IA results will be reviewed and assessed by the DSMC while the participant, Investigator, and Outcome assessor will remain blinded. In addition, a small group of Sponsor personnel will be unblinded to participant level data as well to prepare and support regulatory interactions. Personnel who are unblinded to a critical fraction of the participant level data will subsequently not be allowed to participate in any aspects of the continued trial conduct.

Based on IA3 results, DSMC will make recommendation whether one dose is more favorable with respect to effect while showing no overall difference in safety. The Sponsor will make the final

decision whether to stop the least favorable dose-arm based on DSMC's recommendation and IA results.

Details of the analysis will be included in the SAP, which will be approved prior to the IAs.

9.4.1. Data Safety Monitoring Committee

Table 77 shows the minimal required timing of reviews by the DSMC. Ongoing risk-benefit assessments will be conducted throughout the trial by the independent DSMC. In addition, DSMC will provide a comprehensive review of study safety, tolerability, and PK/PD data, along with a summary of cohort reviews across all cohorts and provide decisions. Further operational details will be prespecified in the DSMC charter.

Table 77: Minimum Timing of the Data Safety Monitoring Committee Data Reviews

Review of data from Study DCR-A1AT-101	The DSMC reviewed all available safety data from the first 3 cohorts of Study DCR-A1AT-101 and affirmed that it is safe to advance the dose level selected by M&S into a multiple dose regimen.
Review of sentinel pair dosing	The DSMC must review the available safety data collected on a sentinel pair of participants, through at least 3 weeks postdose before the remaining participants can be randomized and dosed. The data to be reviewed will be specified in the DSMC charter.
Review of first 10 participants randomized and dosed	The DSMC will review the available safety data collected on the first 10 participants to receive study intervention, through Day 29. The data to be reviewed will be specified in the DSMC charter.
After completion of Cohort 1 (IA1)	The DSMC will review the safety and efficacy data collected through the EOT (Week 24) visit for all participants in Cohort 1.
Occurrence of a potential stopping rule	The DSMC will review and adjudicate all AEs that constitute potential stopping rules, in addition to the cumulative safety data collected on all participants previously dosed. The DSMC will determine whether dosing and enrollment may resume as detailed in Section 7.1.
After completion of Cohort 2 (IA2)	The DSMC will review the safety and efficacy data collected through the EOT (Week 48) visit for all participants in Cohort 2.
Interim Analysis 3 (IA3)	The DSMC will review the safety and efficacy data collected through the Week 24 visit for all participants in Cohort 3.
Additional IA if performed	The DSMC will review the safety and efficacy data collected through additional IA if performed.

Abbreviations: AE: adverse event; DSMC: Data Safety Monitoring Committee; EOT: end of treatment; IA: interim analysis; M&S: modeling and simulation.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Regulatory, Ethical, and Study Oversight Considerations

10.1.1. Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and CIOMS International Ethical Guidelines
- Applicable ICH GCP Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

10.1.2. Financial Disclosure

Investigators and sub-investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit a complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the study and for 1 year after completion of the study.

10.1.3. Informed Consent Process

The Investigator or his/her representative will explain the nature of the study to the participant and answer all questions regarding the study.

Participants must be informed that their participation is voluntary. Participants will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, and the IRB/IEC or study center.

The source document must include a statement that written informed consent was obtained before the participant was enrolled in the study, and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.

A copy of the ICF(s) must be provided to the participant.

A participant who is rescreened is required to sign another ICF.

10.1.4. Data Protection

Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor or Sponsor's designee will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.

The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5. Committee Structure

The external DSMC will be comprised of an independent hepatologist and an independent pulmonologist, both of whom have experience in caring for patients with AATD, and a biostatistician who is also not involved in the study. The DSMC will provide a comprehensive review of study safety and tolerability data, along with a summary of cohort reviews and decisions. Further operational details will be prespecified in the DSMC charter.

The internal Novo Nordisk safety committee will perform ongoing safety surveillance. If new safety signals are identified, these will be evaluated by an internal safety committee. The safety committee may recommend unblinding of any data for further analysis, and in this case an internal trial independent ad hoc group will be established in order to maintain the blinding of the trial personnel.

10.1.6. Dissemination of Clinical Study Data

After de-identification, individual data collected during the trial may be shared with investigators whose proposed use of the data has been approved by the sponsor.

All trial results will be posted to www.clinicaltrials.gov and www.clinicaltrialsregister.eu within 1 year of study completion.

10.1.7. Data Quality Assurance

All participant data relating to the study will be recorded on electronic CRF unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by electronically signing the CRF.

The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.

The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide access to source data documents.

Monitoring details describing methods, responsibilities, and requirements, including handling of non-compliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

The Sponsor or designee is responsible for the data management of this study, including the quality checking of the data.

The Sponsor assumes accountability for actions delegated to other individuals (i.e., Contract Research Organizations).

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator for at least 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

10.1.8. Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator’s site.

Data entered in the eCRF must be consistent with the source documents, or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records. Current medical records must be available.

10.1.9. Study and Site Closure

The Sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Please see Section 7.1 for study stopping rules relating to participant safety. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected, and a study-site closure visit has been performed.

The Investigator may initiate study-site closure at any time, provided there is reasonable cause, and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

1. Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines
2. Inadequate recruitment of participants by the Investigator
3. Discontinuation of further study intervention development

10.1.10. Publication Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.

The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support the publication of multi-center studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with the International Committee of Medical Journal Editors' authorship requirements.

10.2. Clinical Laboratory Tests

The tests detailed in Table 88 will be performed by the central laboratory, with the exception of pregnancy testing and cotinine testing which will be performed locally. Laboratory testing required for immediate safety concerns may also be performed locally at the discretion of the Investigator.

Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 5 of the protocol.

Additional tests that are required for clinical management of the participant may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Investigators must document their review of each laboratory report.

Table 88: Protocol-Required Laboratory Assessments

Laboratory Assessments	Parameters	
Hematology	Red blood cell count hemoglobin hematocrit platelet count reticulocytes mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC)	White blood cell count: lymphocytes, abs and % monocytes, abs and % eosinophils, abs and % basophils, abs and % neutrophils, abs and %

Laboratory Assessments	Parameters	
Clinical Chemistry	alanine aminotransferase (ALT) aspartate aminotransferase (AST) glutamate dehydrogenase (GLDH) gamma glutamyl transferase (GGT) alkaline phosphatase (ALP) bilirubin; total and direct lactate dehydrogenase (LDH) total cholesterol triglycerides	total protein albumin creatine kinase (CK) sodium chloride potassium creatinine urea (BUN) glucose
Routine Urinalysis	Specific gravity, pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase by dipstick Urine protein-to-creatinine ratio (UPCR) Urine albumin-to-creatinine ratio (UACR) Microscopic examination (if blood or protein is abnormal)	
Other safety tests	• Total complement hemolytic activity (CH_{50}): C3a, C5a, and Bb • Serum alpha fetoprotein (AFP) • Coagulation parameters: aPTT, PT, INR • Antidrug antibodies • C-reactive protein • Urine pregnancy test	
Pregnancy testing	Serum or urine human chorionic gonadotropin (hCG) pregnancy test (as needed) (see Schedule of Activities in Section 1.3	
Other Screening Tests	• FSH (as needed in postmenopausal women only) • Urine drug screen (to include, at a minimum: amphetamines, barbiturates, cocaine, opiates, and benzodiazepines) • Serology (HIV antibody, HBsAg, and HCV antibody) • Antimitochondrial antibodies (if suspected for PBC) • HbA1c • Urine cotinine	
Other Tests	• α-2 macroglobulin, haptoglobin, and apolipoprotein A1 (components of non-invasive fibrosis index) • Biomarkers of liver fibrosis (ELF, Pro-C3, tenascin) • Biomarkers of inflammation and liver apoptosis (CK-18, M30, and M65) • Samples for future analyses • Serum AAT (total and Z-AAT) • Liver Z-AAT, PAS-positive AAT globules • Liver histology, including fibrosis	

Abbreviations: aPTT: activated partial thromboplastin time; EGF: epidermal growth factor; FSH: follicle-stimulating hormone; HbA1c: hemoglobin A1c; HBsAg: hepatitis surface antigen; HCV: hepatitis C virus; HIV: human immunodeficiency virus; IFN: interferon; IL: interleukin; INR: international normalized ratio; PBC: primary biliary cholangitis; PT: prothrombin time; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; TNF-α: tumor necrosis factor-alpha.

10.2.1. Samples for Future Analysis

The trial will involve collection of samples to be stored in a central archive for future use. Participants who do not wish to contribute samples for storage may still participate in the trial.

Participants must consent before samples are collected to be stored for future analysis.

The material to be collected is blood (whole blood, plasma and serum) and liver tissue. Blood samples will be collected as indicated in the SoA (Section 1.3).

Liver tissue will be collected from the liver biopsy at week 24, 48 and 96 as applicable.

Samples will be used to improve knowledge and understanding of the biology of the trial disease and related diseases, such as mechanism of action or treatment effect. The biosamples may be transferred to other countries and third parties including commercial vendors such as diagnostics developers for analysis. In case the participant withdraws his/her informed consent for biosamples for future analysis, the monitor must contact the Sponsor trial manager as soon as possible in order to have the samples withdrawn from storage. If the collected biosamples (serum, plasma, liver biopsy samples) will be used in the future, the participant and investigator will not become directly informed by Sponsor about the results.

10.3. Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow up, and Reporting

10.3.1. Definition of AE

AE/TEAE Definition

An AE/TEAE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention. Therefore, an AE/TEAE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.

Events Meeting the AE/TEAE Definition

- Any abnormal laboratory test results or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (i.e., not related to the progression of underlying disease).
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study intervention administration, even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or concomitant medication. Overdose per se will not be reported as an

AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

10.3.2. Definition of SAE

If an event is not an AE per the definition above, it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of the disease).

An SAE is defined as any untoward medical occurrence that, at any dose:

1. **Results in death**
2. **Is life-threatening**

   The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death had it been more severe.

3. **Requires inpatient hospitalization or prolongation of existing hospitalization**

   In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

   Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

4. **Results in persistent disability/incapacity**

   The term "disability" means a substantial disruption of a person's ability to conduct normal life functions.

   This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

5. **Is a congenital anomaly/birth defect**
6. **Other situations:**

   Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

   Examples of such events include invasive or malignant cancers, 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.

10.3.3. Definition of Adverse Event of Special Interest

An AESI is a noteworthy event for the particular product or class of products that a Sponsor may wish to monitor carefully (CIOMS, 2005).

Events Meeting the AESI Definition:

Injection site reaction (ISR):

An ISR is a disorder characterized by an intense adverse reaction (usually immunologic) developing at the site of injection. Potential ISRs will be graded as indicated in Table 99.

Individual signs or symptoms at the injection site (e.g., erythema, swelling, etc.) with an onset of less than 4 hours from the time of study intervention administration will be recorded as AEs (not as ISRs).

Signs or symptoms at the injection site with an onset time of greater than 4 hours postdose will be evaluated according to the CTCAE v. 5.0 criteria for ISR, detailed below. If any of the CTCAE criteria for ISR are met, the event will be recorded as an ISR and graded in accordance with Table 99. If the criteria are not met, individual signs or symptoms will be recorded as AEs and graded in accordance with the intensity categories detailed Section 10.3.4.

Table 99: Grading of Injection Site Reactions, CTCAE v 5.0

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Tenderness with or without associated symptoms (e.g., warmth, erythema, itching)	Pain; lipodystrophy; edema; phlebitis	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death

10.3.4. Recording and Follow-Up of AE and/or SAE

AE and SAE Recording

When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.

The Investigator or designee will then record all relevant AE/SAE information in the CRF.

It is not acceptable for the Investigator to send photocopies of the participant's medical records in lieu of completion of the AE/SAE eCRF page.

There may be instances when copies of medical records for certain cases are requested by the Sponsor. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission.

The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE. The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The intensity of AEs and SAEs will be graded in accordance with CTCAE v. 5.0 grading scale.

Assessment of Causality

The Investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE. A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.

The Investigator will use clinical judgment to determine the relationship. Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration, will be considered and investigated. The Investigator will also consult the Investigator's Brochure and/or Product Information for marketed products in his/her assessment.

For each AE/SAE, the site personnel **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.

There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report. However, **it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the SAE Coordinator.** The Investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.

The causality assessment is one of the criteria used when determining regulatory reporting requirements. The following definitions will be used in assessing causality:

- **Not Related**: Event for which sufficient evidence exists to conclude that the etiology is unrelated to study intervention.
- **Possibly Related**: There is some temporal relationship between the event and the administration of the study intervention, and the event is unlikely to be explained by the participant's medical condition or other therapies.
- **Probably Related**: The temporal relationship between the event and administration of the study intervention is suggestive, and the event is unlikely explained by the participants' medical condition or other therapies.
- **Definitely Related**: The event follows reasonable temporal sequence from the administration of the study intervention, follows a known or suspected response pattern to the study intervention, is confirmed by improvement upon stopping the study intervention, and reappears upon repeated exposure if that occurs.

Follow-up of AEs and SAEs

The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include

additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

If a participant dies during participation in the study or during a recognized follow-up period, the Investigator will provide the Sponsor with a copy of any post-mortem findings, including histopathology.

The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

10.3.5. Reporting of SAEs

Facsimile or electronic transmission of the SAE Report Form is the preferred method to transmit SAE information to the SAE coordinator; however, the SAE Report Form may also be transmitted via email. Appropriate measures such as encryption or leaving out certain identifiers will be enforced to protect the identity of participants as required by local, regional, and national requirements. In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.

Initial notification via telephone does not replace the need for the Investigator to complete and sign the SAE Report Form within the designated reporting time frames.

Contacts for SAE reporting can be found on Page 3 of this document.

10.4. Contraceptive Guidance and Collection of Pregnancy Information

10.4.1. Definitions

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below). If fertility is unclear (e.g., amenorrhea in athletes) and a menstrual cycle cannot be confirmed before the first dose of study intervention, additional evaluation should be considered.

Women in the following categories are not considered WOCBP

1. Premenarchal
2. Premenopausal with one of the following:
    a. Documented hysterectomy
    b. Documented bilateral salpingectomy
    c. Documented bilateral oophorectomy

    For individuals with permanent infertility due to an alternate medical cause other than the above (e.g., müllerian agenesis, androgen insensitivity), Investigator discretion should be applied to determining study entry.

    NOTE: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.

3. Postmenopausal
    - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high FSH level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement is required.
    - Female participants on HRT and whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

10.4.2. Contraception Guidance

Female participants

Women of childbearing potential must agree to use a highly effective method of contraception consistently and correctly as described in Table 1010 during the protocol-defined time frames, as outlined in Section 5.1.

Male participants

Male participants with female partners of childbearing potential are eligible to participate if they agree to ONE of the following during the protocol-defined time frames, as outlined in Section 5.1.

- Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long-term and persistent basis) and agree to remain abstinent
- Agree to use a male condom plus partner use of a contraceptive method with a failure rate of <1% per year as described in Table 1010 when having penile-vaginal intercourse with a WOCBP who is not currently pregnant.
- In addition, male participants must refrain from donating sperm for the duration of the study and for 12 weeks after the last dose of the study intervention.

Male participants with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration during the protocol-defined time frame.

Table 1010: Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Dependent ***Failure rate of <1% per year when used consistently and correctly.***

• Combined (estrogen- and progesterone-containing) hormonal contraception associated with inhibition of ovulation
  • Oral
  • Intravaginal
  • Transdermal
  • Injectable

• Progestogen-only hormonal contraception associated with inhibition of ovulation
  • Oral
  • Injectable

• Sexual abstinence Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.
Highly Effective Methods That Are User Independent [a1]
• Implantable progestogen-only hormonal contraception associated with inhibition of ovulation • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS) • Bilateral tubal occlusion
• Vasectomized partner A vasectomized partner is a highly effective contraception method, provided that the partner is the sole male sexual partner of the WOCBP, and the absence of sperm has been confirmed. If not, an additional, highly effective method of contraception should be used.

Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants participating in clinical studies.

10.4.3. Pregnancy Testing

Pregnancy testing will be conducted approximately every 4 weeks during the study, including F/U.

Pregnancy tests must be performed monthly, at the timepoints specified in the Schedule of Activities (Section 1.3). Home pregnancy tests will be issued to WOCBP for months when no clinic visit is scheduled. Participants will be instructed to contact the clinic staff in case of a positive test.

In addition, a pregnancy test must be performed whenever a menstruation is late or missed, or when pregnancy is otherwise suspected. Home pregnancy tests will be provided to participants and can be resupplied as needed throughout the trial.

10.4.4. Collection of Pregnancy Information

Male participants with partners who become pregnant

- The Investigator will attempt to collect pregnancy information on any male participant's female partner who becomes pregnant while the male participant is in this study.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female participants who become pregnant

- The Investigator will collect pregnancy information on any female participant who becomes pregnant while participating in this study. Information will be recorded on the appropriate form and submitted to the Sponsor within 24 hours of learning of a participant's pregnancy.
- The participant will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the participant and the neonate, and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such. Any post-study pregnancy-related SAE considered reasonably related to the study intervention by the Investigator will be reported to the Sponsor as described in Section 10.3.2. While the Investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.
- Any female participant who becomes pregnant while participating in the study will discontinue study intervention.

10.5. Country-specific Requirements

For Germany only:

In addition to the stopping rules detailed in Section 7.1, the following rule will also apply to sites in Germany:

- 7.1.1.3 Electrocardiogram Findings (Applicable to Germany only)

If QT interval changes as listed below or other clinically significant findings are identified after enrollment, the Investigator or qualified designee will determine if the participant can continue in the study and if any change in participant management is needed.

- Absolute QTc or QTcF > 500 msec and/or
- QTc or QTcF change of > 60 msec from baseline

This review of the ECG printed at the time of collection must be documented. Any new, clinically relevant finding should be reported as an AE.

- 7.1.1.4 CTCAE Grade 4 Adverse Events (Applicable to Germany only)

This study will use NCI CTCAE v. 5.0 to grade AEs, with modifications given for the medical characteristics of the population under investigation.

Study intervention for an individual participant will be permanently discontinued for the occurrence of any Grade 4 AE, regardless of attribution to study intervention, provided that the participant had no signs/symptoms or the signs/symptoms of a Grade 1 or Grade 2 event at baseline.

10.6. Abbreviations

Abbreviation	Description
AAT	alpha-1 antitrypsin
AATD	AAT deficiency
AATLD	Alpha-1 Antitrypsin Deficiency-Associated Liver Disease
AE	adverse event
AESI	AE of special interest
AFP	alpha fetoprotein
ALP	alkaline phosphatase
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
APRI	aspartate aminotransferase to platelet ratio index
Anti-LC	anti-liver cytosol antibody
Anti-SLA/LP	anti-soluble liver/liver pancreas
ASGPR	asialoglycoprotein receptor
ARDS	acute respiratory distress syndrome
ASO	antisense oligonucleotide
AST	aspartate aminotransferase
ATS	American Thoracic Society
AUC	area under the curve
AUC_{last}	area under the concentration time curve at the time of last observation
BMI	body mass index
BP	blood pressure
BSA	body surface area
°C	degrees Celsius
C-F/U	Conditional Follow-up
CK-18	cytokeratin 18
CIOMS	Council for International Organizations of Medical Sciences
CLDQ	Chronic Liver Disease Questionnaire
CL/F	apparent clearance
C_{max}	maximum observed concentration
CONSORT	Consolidated Standards of Reporting Trials
COPD	chronic obstructive pulmonary disease
COVID-19	corona virus disease 2019
CRP	C-reactive protein
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CV%	coefficient of variation (percent)
DCR-S1459	drug substance for this IMP, also known as belcesiran sodium
DCR-A1AT	drug product for this IMP, also known as belcesiran

Abbreviation	Description
DDI	drug-drug interaction
DILI	drug induced liver injury
DLCO	diffusing capacity of the lungs for carbon monoxide
DLT	dose-limiting toxicity
DRF	dose-range-finding
DSMC	Data Safety Monitoring Committee
ECG	electrocardiogram
eCRF	electronic case report form
EFD	embryo-fetal development (study)
e.g.	for example (*exempli gratia*)
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
eGFR	estimated glomerular filtration rate
ELF	enhanced liver fibrosis
EOS	End of Study
EOT	End of Treatment
ERS	European Respiratory Society
ET	early termination
FDA	Food and Drug Administration
FEED	fertility and early embryonic development (study)
FEFv	flow rates
FEV_1	forced expiratory volume in one second
FSH	follicle stimulating hormone
F/U	follow-up
FVC	forced vital capacity
GalNAc	*N*-acetylgalactosamine
GCP	Good Clinical Practice
GGT	gamma glutamyl transferase
HA	Hydrochloric acid
HbA1c	hemoglobin A_{1c}
HBsAg	hepatitis B surface antigen
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HIV	human immunodeficiency virus
hr	hour(s)
HRT	hormonal replacement therapy
IB	Investigator's Brochure
ICF	informed consent form
ICH	The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (formerly International Conference on Harmonisation)

Abbreviation	Description
i.e.	that is (*id est*)
IEC	Independent Ethics Committee
IFN	interferon
IFN-α	interferon alpha
IFN-γ	interferon gamma
IgG	immunoglobulin G
IL-6	interleukin 6
IL-10	interleukin 10
IL-12	interleukin 12
IMP	Investigational Medicinal Product
INR	international normalized ratio
IRB	Institutional Review Board
ISR	injection site reaction
IUD	intrauterine device
IUS	intrauterine hormone-releasing system
IVRS/IWRS	interactive voice/web response system
kg	kilogram(s)
kPa	kilopascal(s)
LFT	liver function test
LSE	liver stiffness evaluation
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
MRE	magnetic resonance elastography
mRNA	messenger ribonucleic acid
M&S	modeling and simulation
NAAT	nucleic acid amplification test
NOAEL	no observed adverse effect level
NSAID(s)	nonsteroidal anti-inflammatory drug(s)
OTC	over the counter
PD	pharmacodynamic(s)
PFT	pulmonary function test(s)
PHT	portal hypertension
PI	prediction interval
PIIINP	amino-terminal propeptide of type III procollagen
PiZZ	autosomal homozygous mutant AAT Z-allele genotype
PK	pharmacokinetic(s)
Pro-C3	N-terminal type III collagen propeptide
PSA	procedural sedation and anesthesia

Abbreviation	Description
PT	prothrombin time
q4w	every 4 weeks
QTcF	QT interval (Fridericia correction)
RNA	ribonucleic acid
RNAi	RNA interference
RT-PCR	reverse transcription polymerase chain reaction
SAD	single ascending dose
SAE	serious adverse event
SAP	Statistical Analysis Plan
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SC	subcutaneous
SD	standard deviation
SERPINA1	the gene that encodes the AAT protein
SGRQ	St. Georges Respiratory Questionnaire
siRNA	small interfering RNA
SNP	single nucleotide polymorphism
SoA	Schedule of Activities
SOC	system organ class
SRC	Safety Review Committee
SUSAR	suspected unexpected serious adverse reactions
$t_{1/2}$	terminal elimination half-life
TEAE	treatment-emergent adverse event
TIMP-1	tissue inhibitor of metalloproteinases 1
TIPS	transjugular intra hepatic portosystemic shunt
TK	toxicokinetic(s)
T_{max}	time to maximum observed concentration
TNF-α	tumor necrosis factor alpha
ULN	upper limit of normal
VC	vital capacity
Vz/F	apparent volume of distribution
WFI	water for injection
WOCBP	woman(en) of childbearing potential
Z-AAT	the mutant AAT protein

10.7. Investigator Signature Page

A Phase 2, Randomized, Double-blind, Placebo--controlled Study Investigating Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Two Dose Levels of Belcesiran in Patients with Alpha-1 Antitrypsin -Associated Liver Disease

Protocol Number: DCR-A1AT-201

Version: 4.0

Date: 02-May-2023

I have read this protocol and agree to conduct this trial in accordance with all stipulations of the protocol and in accordance with Good Clinical Practice (GCP) guidelines, the Declaration of Helsinki, and local regulations (as applicable).

Signature:	**Date:**
Name:	**Title:**
Institution:	**Site Number:**

10.8. Protocol Amendment History

The Protocol Amendment Summary of Changes table for the current amendment is located directly before the Table of Contents.

Description of Change	Brief Rationale	Affected Sections
Study design was updated to include a third cohort, which utilizes a new dose of 50 mg.	The two belcesiran doses included in the clinical protocol (210 mg and 50 mg) are predicted to result in a range of PD response that would allow for adequate characterization of belcesiran dose-exposure-response in patients with AATLD.	Section 1.1 Section 1.2 Section 1.3 Section 2.1 Section 2.2.5 Section 3 Section 4.1 Section 4.3 Section 6.1 Section 9.1 Section 9.3 Section 9.4
An optional extended treatment period of quarterly dosing is added for Cohorts 1 and 2.	The rationale for exploring an alternative dosing regime is out of consideration for patient burden. For the high dose explored, simulations suggest that reducing dose frequency after steady state would only have a minor effect on the PD response.	Section 1.2 Section 1.3 Section 4.1 Section 7.1.1
The follow-up period is no longer conditional. Each cohort will have a fixed follow-up period.	This update ensures that blinded personnel remain blinded to subject treatment assignments throughout the follow-up period.	Section 1.1 Section 1.2 Section 1.3 Section 4.1 Section 4.2 Section 4.4 Section 6.3 Section 8.1
The number of subjects to be enrolled decreased to 46 subjects.	Updates based on the revised study design.	Section 1.1 Section 4.1 Section 9.1

Description of Change	Brief Rationale	Affected Sections
Baseline serum AAT will now be the predose value at D1 instead of the mean of the values from screening and D1	To simplify the serum AAT baseline calculation algorithm.	Section 8.1.1 Section 9.1
The maximum age for inclusion in the study is changed from 70 years to 75 years	To include subjects aged 70 through 75.	Section 1.1 Section 5.1
Participants with liver fibrosis score of F1 on the METAVIR scoring system now included	To facilitate recruitment and to explore the effects of belcesiran in this patient population	Section 1.1 Section 4.1 Section 5.1 Section 8.1 Section 9.3.1
Inclusion criteria for serum AAT protein concentration > 10 mg/dL during screening is removed.	Since PiZZ confirmed AATLD patients are expected to have a protein concentration > 10mg/dL, the criterion was removed to decrease patient burden and facilitate recruitment.	Section 1.1 Section 5.1
Post-bronchodilator inclusion criteria updated to include participants with FEV_1 >45% of predicted at screening instead of the previous FEV_1 >60%	The inclusion of patients on augmentation therapy requires bronchodilator inclusion criteria to be lowered.	Section 1.1 Section 5.1
Participants on augmentation therapy are now allowed to enroll	To include subjects that are prescribed augmentation therapy to facilitate recruitment and assess the effects of belcesiran in this patient population	Section 1.1 Section 2.3.1.2 Section 2.3.3 Section 5.1 Section 5.2 Section 6.5.1 Section 7.1.1
Removed augmentation therapy as a treatment related to exacerbations of pre-existing emphysema. Investigators who start a participant on augmentation therapy must have experience with the therapy.	Updated to provide further detail and clarification.	Section 2.3.4 Section 6.5.1 Section 7.1.1.1

Description of Change	Brief Rationale	Affected Sections
Smoking inclusion criteria updated, and cotinine testing a e at Investigator's iscretion	To ensure adequate confirmation that patient is not smoking.	Section 1.1 Section 1.3 Section 5.1 Section 8.2.1 Section 10.2
WOCBP are now allowed to enroll • Pregnancy testing is added to all Cohorts • At home pregnancy testing allowed for months where participants do not come to site • Contraception wording updated	To include WOCBP. Inclusion of WOCBP will require more pregnancy testing. We are allowing for home pregnancy tests in between site visits to ensure that monthly pregnancy testing takes place.	Section 1.1 Section 1.3 Section 4.2 Section 5.1 Section 8.3.5 Section 10.2 Section 10.4
Exclusion criteria was clarified to further define history of chronic liver disease and history of a severe exacerbation	Updated for clarity.	Section 1.1 Section 5.2
Risks related to potential off-target effects was updated.	Updated based on additional data.	Section 2.3.1.3
Exclusion criteria around alcohol consumption and drug abuse were clarified	Updated for clarity.	Section 5.2 Section 5.3 Section 7.1 Section 8.5
Cytokines and some complement tests are removed.	Cytokines and complements were reviewed by an independent rheumatologist for the DCR-PHXC program. This review revealed no interpretable signal. In addition, there have been no AEs suggestive of system immune activation across the Dicerna program.	Section 1.3 Section 2.2.5 Section 9.3.1 Section 10.2
History of rapid decline in pulmonary function as assessed by Investigator was added as an exclusion.	Exclusion was added to allow for investigator discretion when determining eligibility.	Section 1.1 Section 5.2

Description of Change	Brief Rationale	Affected Sections
Added exclusions for specific heart related conditions and malignancy within the last 90 days	Exclusion was a e to ensure the patient's health is stable when determining eligibility.	Section 5.2
Removed the restrictions around acetaminophen/paracetamol, steroid, and NSAID use	Exclusion was removed to allow for investigator discretion when determining eligibility.	Section 5.2 Section 6.5
Exclusion criteria around HbA1c, triglycerides, white blood cells, and neutrophils lab tests and blood pressure were removed	Exclusion was removed to allow for investigator discretion when determining eligibility.	Section 5.2
Physical examination assessments further defined and allowed to be focused at many visits. Height and weight were also separated into their own assessment in the schedule of assessments.	For clarity and to decrease site/patient burden	Section 1.3 Section 8.2.2
CLDQ and SGRQ added	Added to collect quality of life data on enrolled subjects.	Section 1.3 Section 5.2 Section 8.1.6 Section 8.1.7
Blood biomarker assessments further defined	For clarity between blood biomarkers of inflammation and fibrosis	Section 1.3 Section 8.1.3 Section 10.2
Samples for future research assessments further defined.	Revised to include details around collection of future use samples for clarity.	Section 1.3 Section 4.2 Section 8.1.5 Section 10.2
Additional parameters were added to the liver histology evaluation	Updated for clarity.	Section 8.1.2.3 Section 10.2
Measures of liver fibrosis were clarified	Updated for clarity.	Section 8.1 Section 10.2
An optional liver biopsy is added at 96 weeks.	Added based on updated study design.	Section 1.1 Section 1.2

Description of Change	Brief Rationale	Affected Sections
		Section 1.3 Section 4.1 Section 4.2 Section 5.1 Section 8.1.2 Section 10.2.1
Fasting requirements were clarified	Updated for clarity.	Section 1.3 Section 8.1 Section 8.2.6
Definition of clinically significant abnormal laboratory findings is removed	Updated for clarity.	Section 8.2.6
FibroScan expectations were updated and are only to be conducted if sites have access to the technology.	Up ate for clarity an to account for site's that o not have access to FibroScan equipment.	Section 1.3 Section 8.1.4.1
MRE assessments are only to be conducted if sites have access to the technology.	To harmonize country specific amendments and to account for site's that o not have access to MRE equipment or technology.	Section 1.3 Section 8.1.4.2
Some visits can be conducted via phone rather than in-clinic.	Updated to lessen patient burden.	Section 1.3 Section 8.1 Section 8.2
The primary endpoint was updated from change from baseline in serum AAT protein concentration over time to changes from baseline to Week 24 (Cohort 1) and Week 48 (Cohort 2) in serum AAT protein concentrations.	Updated to define the time over which the primary endpoint would be analyzed.	Section 1.1 Section 3 Section 9.1
A primary objective/endpoint is added (for cohort 3 only) to characterize the PD of belcesiran in patients with AATLD as measured by change from baseline to Week 24 in serum and liver Z-AAT protein levels.	Added based on updated study design.	Section 1.1 Section 3 Section 9.1
Secondary and exploratory endpoints are amended to include the change from baseline up to Week 96 and include clarity around assessments being done.	Added based on updated study design.	Section 1.1 Section 3

Description of Change	Brief Rationale	Affected Sections
		Section 9.1
The exploratory endpoint of change from baseline in blood-based biomarkers of inflammation is removed.	Removed based on updated study design.	Section 1.1 Section 3 Section 9.1
Injection Site Inspection added to all Treatment Period SOAs	To clarify that this is expected after every dosing	Section 1.3
Statistical analyses are changed to accommodate changes to the study design and endpoints.	To reflect the changes of study design and ensure sample size for Cohort 3 is powered and primary analysis model is pre-specified.	Section 4.1 Section 9
Added F1 fibrosis type to the randomization stratification factor.	To include patients with F1 fibrosis type in this study.	Section 9.1
COVID testing is removed as a requirement.	COVID testing was updated to be performed as necessary.	Section 1.3 Section 6.5.2 Section 8.1.8 Section 10.2
Screening windows are updated.	To decrease site and patient burden when scheduling screening assessments.	Section 1.3
Nonclinical background updated • Includes updates to off-target effects	To reflect changes to the Investigator Brochure v5.0 made with more recent information	Section 2.2.3 Section 2.3
Clinical background updated • Includes updates to AAT knockdown	To reflect changes to the Investigator Brochure v5.0 made with more recent data from the DCR-A1AT-101 study	Section 2.2.4 Section 2.3.1.1 Section 2.3.1.2 Section 4.3
Language regarding risks related to knockdown was revised	To include more recent data from the DCR-A1AT-101 study	Section 2.3.1.2
AE/SAE risks updated	To include more recent data from the DCR-A1AT-101 study	Section 2.3.3 Section 2.3.1.1
Clinical data from DCR-A1AT-101 study was updated	To include more recent data from the DCR-A1AT-101 study	Section 2.2.4

Description of Change	Brief Rationale	Affected Sections
TEAE defined	Updated for clarity	Section 10.3.1
Reporting of AEs/SAEs was updated	Updated for clarity	Section 8.3.1
Instructions on changes in PK timepoints were removed	Updated for clarity	Section 8.5
Rescreening of screen failed patients was clarified	Updated for clarity.	Section 5.4
Home dosing was included as an option if the participant is unable to come to the study site	Updated to lessen patient burden.	Section 6.4
Stopping rules were updated	Updated to provide further detail and clarification.	Section 7
Maximum blood volume limits were updated	To reflect changes in laboratory assessments.	Section 8
Editorial changes for clarity.	Updates made for clarity	Throughout
Mention of the long term, open label extension study has been removed from the protocol	Administrative update.	Section 6.7
Protocol title updated	To reflect changes made throughout the protocol	Title page Sponsor Signature Page Section 1.1 Section 10.6
Study phase updated to 2 from 2a	To reflect that the data from this study will be used for both safety and efficacy parameters	Title page
Updated Sponsor and CRO contacts	Administrative Changes in Sponsor/CRO personnel.	Sponsor Signature Page Medical Monitor and Pharmacovigilance Contact Information

Amendment 1 (version 2.0), 04-Feb-2021

The 03-Sep-2020 amendment was principally undertaken to include the dosing regimen, to specify that C-reactive protein will be measured at the same time as serum AAT, and to clarify procedures affected by and/or implemented to address the COVID-19 pandemic.

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

The table below summarizes all changes in Version 2.0 from Version 1.0.

Description of Change	Brief Rationale	Affected Sections
Updated dosage and other information relating to the IMP • Replace "DCR-A1AT" with the a opte name "belcesiran" • Specified the dosage of belcesiran to be administered in the study (210 mg [1.2 mL injection volume] once every 4 weeks). • Clarified that the dosage of belcesiran is expressed in terms of the free-acid form of the drug substance. • Removed lot number of IMP from table of study interventions	• The INN was recently adopted • The dosage was determined by modeling and simulation of PK/PD data from an interim analysis of Study DCR-A1AT-101 and was affirmed by the SRC and DSMC. • The US Pharmacopeia states that when the active ingredient in a drug product is a salt, the strength should be expressed in terms of the active moiety rather than salt strength equivalent. • More than one lot number may be used in the study	1.1 Synopsis 1.2 Schema 2.2.5 Summary 2.3.3 Risk Assessment 4.1 Overall Study Design 4.3 Justification for Dose 6.1 Study Intervention(s) Administered 6.1.1 Dose Level 6.2 Preparation/Handling/ Storage/Accountability Belcesiran name change - throughout the protocol
Provided separate Schedules of Activities for Cohort 1 and Cohort 2	Ease of interpretation	1.3 Schedules of Activities
Specified that C-reactive protein will be measured at the same time as all serum AAT protein concentrations, and that any AAT concertation that is accompanied by a CRP value above the upper limit of normal will be repeated.	C-reactive protein and A1AT are both acute-phase proteins released into the plasma in response to inflammatory stimuli. Concomitant assessment of CRP and A1AT will allow discern between elevations in A1AT due to inflammatory stimuli or to study intervention.	1.3 Schedules of Activities 5.4.1 Repeated Screening Tests 8.1.1.1 Calculation of Baseline Serum A1AT Concentration 8.1.1.2 Conditional Follow-up

Description of Change	Brief Rationale	Affected Sections
Addressed the impact of COVID-19: • All prospective participants must have a negative test for SARS-CoV-2 at the time of randomization • Any participant testing positive for SARS-CoV-2 will be discontinued from study intervention • Telehealth and/or home nursing visits may be employed if participants are unable to return to the site due to COVID-19 restrictions • Vaccine administration should be separated by 7 days from administration of study intervention	• To ensure that randomized participants do not have COVID-19 • To decrease the risk for decline in pulmonary function that could arise from concomitant infection with SARS-CoV-2 • To enable participant to continue study participant during the pandemic • To enable differentiation of potential AEs due to the vaccine from those due to study intervention	1.3 Schedules of Activities 4.1 Overall Study Design 5.2 Exclusion Criteria 6.5.1 Rescue Medication 6.5.2 COVID-19 Vaccine 7.1.1.2 Positive SARS-CoV-2 Test 7.2 Participant Discontinuation/Withdrawal from Study 10.2 Clinical Laboratory Tests 10.5 Abbreviations
Added a statement to note that any participant with a serum AAT protein concentration that has not returned to ≥ 80% of Baseline by Week 48 of con itional follow-up will be followed in a separate study.	To ensure that long-term follow-up of participants occurs within a sponsored study protocol.	1.1 Synopsis 1.3 Schedules of Activities 2.2.4 Clinical Overview 4.1 Overall Study Design 4.4 End of Study Definition 8.1.1.2 Conditional Follow-up 8.3.1 Time Period and Frequency for Event Assessment
Updated clinical laboratory tests to be performed during • the conditional follow-up period • Screening	• Increased participant safety • Removed tests that are now scheduled to be assessed on an ongoing basis and corrected typographical errors in version 1.0	1.3 Schedules of Activities 8.1.1.2 Conditional Follow-up 8.2.1 Screening Assessments 10.2 Clinical Laboratory Tests
Updated description of indirect, noninvasive fibrosis blood biomarker panel, and added 3 analytes to be collected.	The trademarked LabCorp FibroSure test will not be performed as previously specified. The Sponsor will calculate the fibrosis score from the individual components of the panel.	8.1.3.1 Biochemical Fibrosis Index 10.2 Clinical Laboratory Tests

Description of Change	Brief Rationale	Affected Sections
Added section describing the determination of hepatic Z-AAT protein content	Not previously included	8.1.2.4 Hepatic Z-AAT Content
Stated that SAEs will be collected until 30 days after the last day of study participation or until serum AAT protein concentration is ≥ 80% of baseline on 2 consecutive visits, whichever is longer.	To ensure that participants are followed for occurrence of SAEs for a sufficient period of time.	1.3 Schedules of Activities 8.3.1 Time Period and Frequency for Event Assessment
Updated DLCO criteria in the individual participant stopping rules. The absolute change in DLCO was lowere to ≥ 6.5 m /min/mmHg from ≥ 8 mL/min/mmHg	Increased participant safety	7.1.1.3 Decline in Pulmonary Function
Clarified that the duration of the conditional follow-up period is up to 48 weeks, not 12 months	Greater accuracy	1.1 Synopsis 1.3 Schedules of Activities 2.2.4 Clinical Overview 4.1 Overall Study Design 4.4 End of Study Definition 8.1.1.2 Conditional Follow-up
Clarified that the intensity of all AEs will be graded in accordance with CTCAE v 5.0	A "mil /mo erate/severe" rating scale is not needed in addition to CTCAE grading	10.3.4. Recording and Follow-Up of AE and/or SAE
Added a statement of statistical power	Previously not included	1.1 Synopsis 9.1 Randomization and Sample Size
Updated language to specify the nature, number, and timing of the interim analyses	To clarify that one interim futility analysis will be performed and that additional analyses to support composite endpoint determination may be performed.	9.4 Interim Analyses
Added steatosis scores as an endpoint	Previously missing in the tables of Objectives and Endpoints	1.1 Synopsis 3 Objectives and Endpoints
Removed determination of PK in urine	No longer needed in Phase 2	1.1 Synopsis 1.3 Schedules of Activities 3 Objectives and Endpoints 4.1 Overall Study Design 8.5 Pharmacokinetics

Description of Change	Brief Rationale	Affected Sections
		8.5.1 Metabolite Identification 9.3.2.1 Pharmacokinetic Analyses
Specified that blood pressure and ECG readings are to be conducted in the sitting rather supine position and that only 5 minutes of rest (not 10 minutes) are required	Consistency with clinical practice	5.2 Exclusion Criteria 8.2.3 Vital Signs Measurements 8.2.4 Electrocardiograms
Removed clarification that heart rate will be taken from the ECG when the ECG and vital signs are recorded at the same time and added ventricular rate and RR to the list of parameters to be recorded via ECG.	To better align with electronic data outputs	1.3 Schedules of Activities 8.2.3 Vital Signs Measurements 8.2.4 Electrocardiograms
Updated interval for rescreening due to exacerbations of underlying lung disease from 2 to 3 months	To ensure that participants have stable lung function at the time of enrollment.	5.2 Exclusion Criteria 5.4 Screen Failures
Removed Medical Monitor approval of restricted concomitant medications	Ad hoc approval is inconsistent with intent of restrictions	6.5 Concomitant Medications
Updated units of measure for DLCO to mL/min/mmHg	Consistency across protocol	8.2.5.2 Diffusion Capacity of the Lungs for Carbon Monoxide
Removed language requesting that the Investigator to make every effort to contact the medical monitor prior to unblinding in case of medical emergency.	The decision to unblind in an emergency belongs solely to the Investigator.	6.3 Measures to Minimize Bias: Randomization and Blinding
Deleted Heading *8.8 Biomarkers*. Promoted Heading *8.8.1 Immunogenicity Assessments* to 8.8	Consistency with protocol template	8.8 ~~Biomarkers~~ Immunogenicity Assessments
Updated the acronyms for alpha-1 antitrypsin deficiency and alpha-1 antitrypsin deficiency-associated liver disease	To conform with new style guide	Throughout the protocol
Administrative updates	Updated personnel, updated Sponsor corporate address, updated reference and abbreviation lists, and other minor corrections	Cover Page, Sponsor Signature Page, References, Abbreviations, and throughout the protocol

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