ORIGINAL ARTICLE

Fazirsiran for Liver Disease Associated with Alpha$_1$-Antitrypsin Deficiency

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ABSTRACT

BACKGROUND

Alpha$_1$-antitrypsin (AAT) deficiency results from carriage of a homozygous *SERPINA1* "Z" mutation (proteinase inhibitor [PI] ZZ). The Z allele produces a mutant AAT protein called Z-AAT, which accumulates in hepatocytes and can lead to progressive liver disease and fibrosis. This open-label, phase 2 trial investigated the safety and efficacy of fazirsiran, an RNA interference therapeutic, in patients with liver disease associated with AAT deficiency.

METHODS

We assigned adults with the PI ZZ genotype and liver fibrosis to receive fazirsiran at a dose of 200 mg (cohorts 1 [4 patients] and 2 [8 patients]) or 100 mg (cohort 1b [4 patients]) subcutaneously on day 1 and week 4 and then every 12 weeks. The primary end point was the change from baseline to week 24 (cohorts 1 and 1b) or week 48 (cohort 2) in liver Z-AAT concentrations, which were measured by means of liquid chromatography–mass spectrometry.

RESULTS

All the patients had reduced accumulation of Z-AAT in the liver (median reduction, 83% at week 24 or 48). The nadir in serum was a reduction of approximately 90%, and treatment was also associated with a reduction in histologic globule burden (from a mean score of 7.4 [scores range from 0 to 9, with higher scores indicating a greater globule burden] at baseline to 2.3 at week 24 or 48). All cohorts had reductions in liver enzyme concentrations. Fibrosis regression was observed in 7 of 15 patients and fibrosis progression in 2 of 15 patients after 24 or 48 weeks. There were no adverse events leading to trial or drug discontinuation. Four serious adverse events (viral myocarditis, diverticulitis, dyspnea, and vestibular neuronitis) resolved.

CONCLUSIONS

In this small trial, fazirsiran was associated with a strong reduction of Z-AAT concentrations in the serum and liver and concurrent improvements in liver enzyme concentrations. (Funded by Arrowhead Pharmaceuticals; AROAAT-2002 ClinicalTrials.gov number, NCT03946449.)

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The gene *SERPINA1* encodes alpha$_1$-antitrypsin (AAT), which is primarily synthesized in hepatocytes and secreted into the circulation, where it protects lung tissue through its antiprotease activity.[1] AAT deficiency is caused by mutations in *SERPINA1*, leading to loss-of-function pulmonary disease and gain-of-function liver disease. A total of 95% of severe cases of AAT deficiency are due to homozygous substitution of a single amino acid, Glu342Lys (proteinase inhibitor [PI] ZZ), which produces a misfolded and poorly secreted AAT protein called Z-AAT, leading to retention of Z-AAT in the liver and a deficiency of AAT in serum. Serum deficiency predisposes patients to emphysema. In the liver, mutant proteins polymerize and persist in the endoplasmic reticulum of hepatocytes as inclusions that are positive on periodic acid–Schiff staining with diastase digestion (PAS-D), a histologic hallmark of liver disease associated with AAT deficiency. Z-AAT accumulation triggers endoplasmic reticulum stress, hepatocellular injury, inflammation, and eventually fibrosis,[2] which can progress to cirrhosis or portal hypertension and lead to hepatic decompensation or hepatocellular carcinoma.[3,4] The lifetime risk of cirrhosis may be 20 to 40% among persons with liver disease associated with AAT deficiency.[4-7] Without liver transplantation, the prognosis for patients with end-stage liver disease is poor.[8]

In adults, liver disease associated with AAT deficiency usually manifests in the fourth or fifth decade of life. A third of adults with the PI ZZ genotype may have clinically significant liver fibrosis, according to studies that used histologic assessments and measurements of liver stiffness.[2,9] Z-AAT accumulation has been correlated with liver fibrosis, a finding that suggests that reducing Z-AAT production may improve hepatic phenotypes.[9] Moreover, elevated concentrations of liver enzymes (alanine aminotransferase [ALT] and γ-glutamyltransferase), portal inflammation, and hepatocellular degeneration have been associated with fibrosis severity.[9] There is currently no specific treatment for liver disease associated with AAT deficiency.

RNA interference (RNAi) is a naturally occurring cellular mechanism that regulates gene expression. Fazirsiran (previously ARO-AAT) is an investigational RNAi therapeutic that contains a synthetic, double-stranded, small interfering RNA duplex conjugated to *N*-acetylgalactosamine, which binds to the hepatocyte asialoglycoprotein receptor to facilitate endosomal uptake and intracellular delivery.[10] (For the structure of fazirsiran, see the Methods section in the Supplementary Appendix, available with the full text of this article at NEJM.org.) Fazirsiran causes degradation of AAT and Z-AAT messenger RNA, thus reducing both AAT and Z-AAT protein synthesis in hepatocytes.

Transgenic mice expressing human Z-AAT recapitulate human liver disease associated with AAT deficiency.[11,12] In this mouse model, fazirsiran treatment effectively reduced serum Z-AAT concentrations, intrahepatic Z-AAT accumulation, inflammation, and endoplasmic reticulum and mitochondrial injury and prevented activation of fibrosis-associated genes.[10] A phase 1 trial involving healthy volunteers showed an adequate safety profile[10] and informed the dose for this phase 2 trial (AROAAT-2002) that assessed the safety, pharmacodynamics, and efficacy of fazirsiran in patients with liver disease associated with homozygous (PI ZZ) AAT deficiency.

A Quick Take is available at NEJM.org

METHODS

TRIAL DESIGN AND PATIENTS

We conducted a multicenter, phase 2, open-label trial that enrolled adults (18 to 75 years of age) with the PI ZZ genotype and F1 to F3 liver fibrosis (Metavir staging or equivalent) on the basis of a local pathological reading at screening. Patients who were determined to have F4 fibrosis (cirrhosis) on the basis of a local pathological reading, who used alcohol regularly, or who smoked (daily cigarette use for >12 months) were excluded. Other key exclusion criteria were ALT and aspartate aminotransferase (AST) concentrations of more than 250 U per liter, an estimated glomerular filtration rate of less than 60 ml per minute, a postbronchodilation forced expiratory volume in 1 second (FEV_1) of less than 65% in patients not receiving AAT augmentation therapy, and a postbronchodilation FEV_1 of less than 45% in patients receiving AAT augmentation therapy. Patients with nonalcoholic steatohepatitis or nonalcoholic fatty liver disease were permitted to participate in the trial if the disease was stable and did not pose a substantial threat to participation.

Patients were sequentially enrolled into three cohorts (Fig. S1 in the Supplementary Appendix), with cohort 1 (4 patients) and cohort 2 (8 pa-

Table 1. Characteristics of the Patients at Baseline.*

Characteristic	Fazirsiran, 200 mg		Fazirsiran, 100 mg: Cohort 1b (N=4)	Total (N=16)
	Cohort 1 (N=4)	Cohort 2 (N=8)		
Age — yr				
Mean	45±17	55±14	55±10	52±14
Range	20–56	24–66	41–65	20–66
Male sex — no. (%)	4 (100)	7 (88)	3 (75)	14 (88)
Weight — kg	87±14	77±14	83±17	81±14
Body-mass index†	26.3±3.2	24.1±4.7	27.5±4.1	25.5±4.2
Fibrosis stage — no. (%)‡				
F0	0	0	1 (25)	1 (6)
F1	0	1 (12)	1 (25)	2 (12)
F2	1 (25)	4 (50)	1 (25)	6 (38)
F3	1 (25)	3 (38)	0	4 (25)
F4	2 (50)	0	0	2 (12)
Not evaluable	0	0	1 (25)	1 (6)
Receiving AAT augmentation therapy — no. (%)	1 (25)	4 (50)	1 (25)	6 (38)
Percentage of predicted FEV_1	54±NA	63.3±10.5	70±NA	62.8±9.6
Not receiving AAT augmentation therapy — no. (%)	3 (75)	4 (50)	3 (75)	10 (62)
Percentage of predicted FEV_1	98.7±14.5	89.0±12.8	111±9.8	98.5±14.7

* Plus–minus values are means ±SD. Percentages may not add up to 100 because of rounding. AAT denotes $alpha_1$-antitrypsin, FEV_1 forced expiratory volume in 1 second, and NA not applicable.
† The body-mass index is the weight in kilograms divided by the square of the height in meters.
‡ Fibrosis stage was determined by central reading. F0 denotes no fibrosis, F1 portal fibrosis without septa, F2 portal fibrosis with few septa, F3 numerous septa without cirrhosis, and F4 cirrhosis.

tients) receiving 200 mg of fazirsiran and cohort 1b (4 patients) receiving 100 mg. Cohort 1b was added during the course of the trial to evaluate dose response. Fazirsiran was administered subcutaneously on day 1, week 4, and every 12 weeks thereafter. Paired liver-biopsy samples were collected according to standardized methods at baseline and after baseline (week 24 for cohorts 1 and 1b and week 48 for cohort 2). Two core liver-biopsy samples were collected per patient per visit for assessments of liver Z-AAT concentration (snap frozen) and histopathological characteristics (formalin fixed). Patients in cohorts 1 and 1b could continue into an extension period after completing the trial visit at week 24, and those in cohort 2 could enter the extension period after completing the trial visit at week 48.

END POINTS

The primary end point was the change from baseline over time in liver Z-AAT concentrations (total, soluble fraction, and insoluble fraction) as measured with the use of liquid chromatography–tandem mass spectrometry that was sensitive and specific for a unique signature peptide containing the Z allele amino acid mutation. (For details, see the Methods section in the Supplementary Appendix.)

Pharmacodynamics were evaluated by means of serum Z-AAT concentration (liquid chromatography–tandem mass spectrometry). All histologic assessments (hematoxylin and eosin, trichrome, and PAS-D) were centrally read with the use of semiquantitative scales and adjudicated by three histopathologists who were not aware of the cohort assignments and the time point of

biopsies. A PAS-D histologic scale was used to complement the liquid chromatography–tandem mass spectrometry method and to measure the degree of global portal-tract involvement, zone 1 periportal hepatocyte involvement, and PAS-D zonal location, with higher scores indicating a higher globule burden (Table S1).

Liver fibrosis was assessed with the use of Metavir staging (F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis). We also assayed serum Pro-C3, a neoepitope of propeptide of type III collagen and a biomarker of fibrosis stage (see the Methods section in the Supplementary Appendix for details of the assay). Key histologic measures of liver disease associated with AAT deficiency included portal inflammation, interface hepatitis, hepatocyte cell death, and lobular inflammation. Biomarkers of liver health included serum levels of liver enzymes (ALT, AST, and γ-glutamyltransferase) and findings on FibroScan (Echosens), an ultrasound-based assessment of liver stiffness (measured in kPa) also known as vibration-controlled transient elastography.

Safety was evaluated on the basis of the incidence and severity of adverse events that emerged or worsened after the first administration of fazirsiran and on the basis of changes in laboratory measurements and pulmonary function (FEV_1 and diffusing capacity of the lungs for carbon monoxide adjusted for hemoglobin concentration [$DLCO_{hbg}$]).

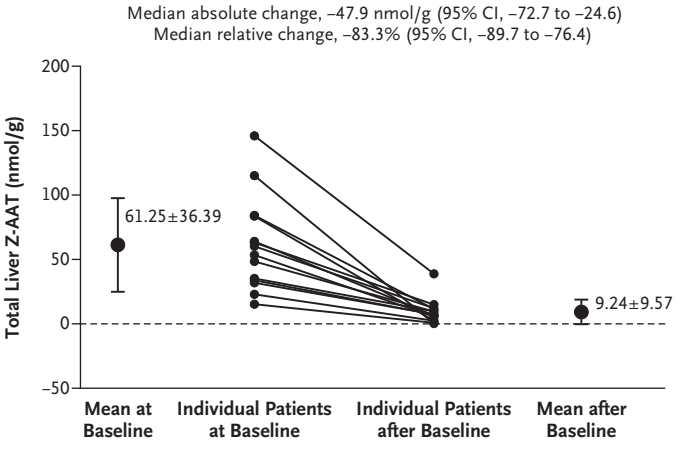

Figure 1. Effect of Fazirsiran Treatment on Liver Z-AAT Concentration at Week 24 or 48.

Shown are concentrations of alpha$_1$-antitrypsin Z-mutant protein (Z-AAT) in the liver for individual patients at baseline and after baseline (at week 24 in cohorts 1 and 1b and at week 48 in cohort 2). Two patients in cohort 2 had insufficient liver-biopsy samples for analysis by means of liquid chromatography–tandem mass spectrometry; data from these two patients were excluded from this summary. One patient in cohort 2 had a post-baseline value below the detection limit; a value of lower limit of detection divided by 2 was used for this summary. Confidence intervals for median changes are distribution-free 95% confidence intervals. The I bars represent standard deviations.

TRIAL OVERSIGHT

This trial was conducted at four centers in Austria, Germany, and the United Kingdom. The ethics committee at each participating center approved the protocol (available at NEJM.org), and the trial was conducted in accordance with the principles of the Declaration of Helsinki, the International Council for Harmonisation Good Clinical Practice guidelines, and applicable regulatory requirements. All the patients provided written informed consent before enrollment.

Arrowhead Pharmaceuticals designed and conducted the trial and collected and analyzed the data. All the authors had access to and were involved in the interpretation of the data and collaborated in the preparation of the manuscript. The first author wrote the first draft and all revisions of the manuscript, with assistance from all the coauthors. All the authors made the decision to submit the manuscript for publication, reviewed and approved the final version, and vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol. The authors and their institutions had confidentiality agreements with Arrowhead Pharmaceuticals.

STATISTICAL ANALYSIS

The sample size was not determined on the basis of statistical hypothesis testing. Analyses for efficacy, pharmacodynamics, and safety end points were based on findings in all enrolled patients who received at least one dose of fazirsiran. Missing data were not imputed.

Descriptive statistics were provided for all end points mentioned above. The change from baseline and percentage change from baseline in total liver Z-AAT concentration were quantified with medians and corresponding distribution-free 95% confidence intervals. For details, see the Methods section in the Supplementary Appendix.

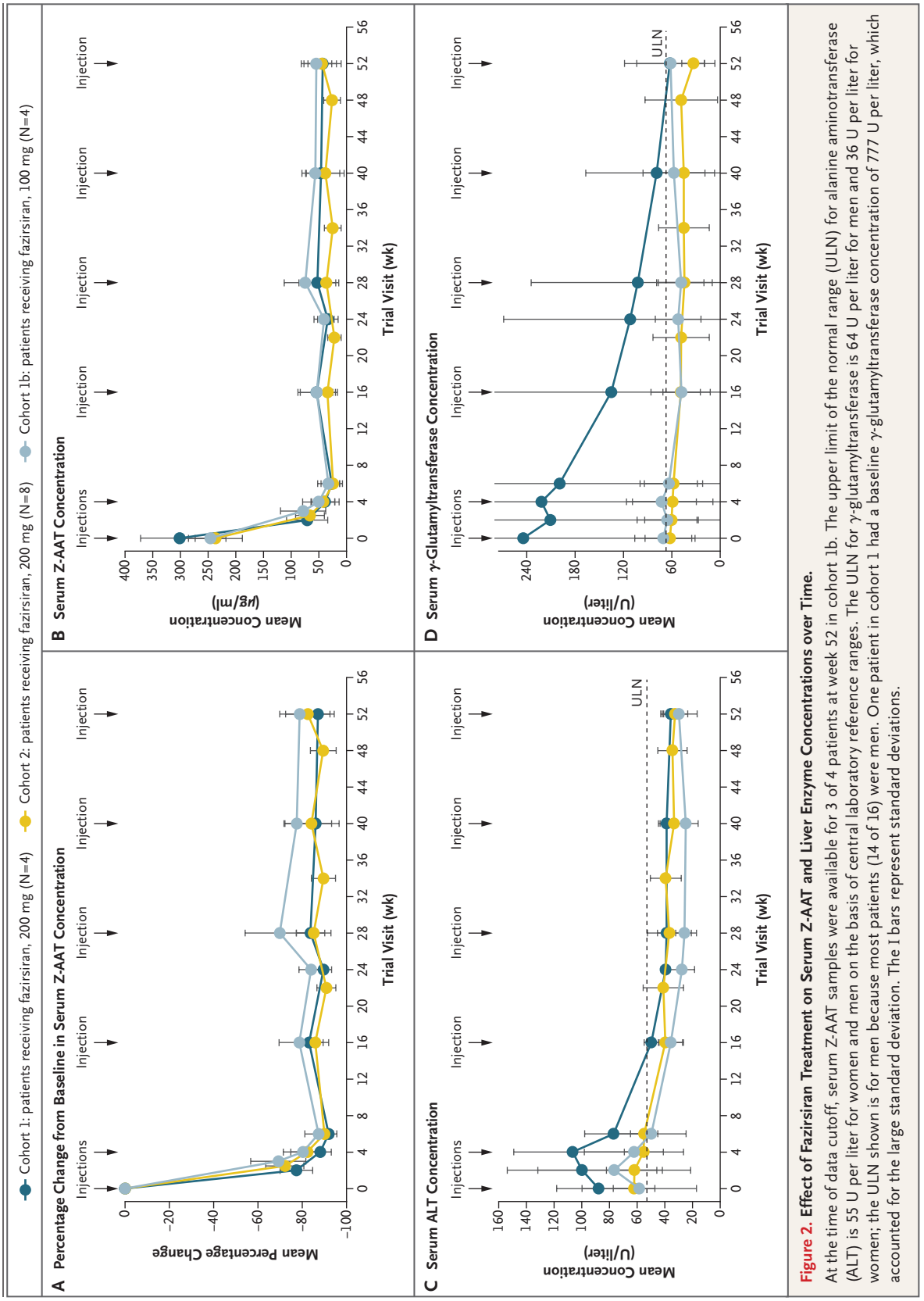

Figure 2. Effect of Fazirsiran Treatment on Serum Z-AAT and Liver Enzyme Concentrations over Time.

At the time of data cutoff, serum Z-AAT samples were available for 3 of 4 patients at week 52 in cohort 1b. The upper limit of the normal range (ULN) for alanine aminotransferase (ALT) is 55 U per liter for women and men on the basis of central laboratory reference ranges. The ULN for γ-glutamyltransferase is 64 U per liter for men and 36 U per liter for women; the ULN shown is for men because most patients (14 of 16) were men. One patient in cohort 1 had a baseline γ-glutamyltransferase concentration of 777 U per liter, which accounted for the large standard deviation. The I bars represent standard deviations.

Table 2. Summary of Safety and Adverse Events.*

Event	Fazirsiran, 200 mg (N=12)	Fazirsiran, 100 mg (N=4)	All (N=16)
		number (percent)	
Any adverse event	11 (92)	4 (100)	15 (94)
Adverse events in 2 or more patients			
Arthralgia	3 (25)	1 (25)	4 (25)
Blood creatine kinase increase	3 (25)	1 (25)	4 (25)
Blood glucose increase	2 (17)	0	2 (12)
Back pain	2 (17)	1 (25)	3 (19)
Chest pain	2 (17)	1 (25)	3 (19)
Diarrhea	3 (25)	0	3 (19)
Dizziness	1 (8)	2 (50)	3 (19)
Dyspnea	2 (17)	1 (25)	3 (19)
Fatigue	1 (8)	1 (25)	2 (12)
Headache	2 (17)	1 (25)	3 (19)
Injection-site reaction	1 (8)	1 (25)	2 (12)
Nasopharyngitis	2 (17)	1 (25)	3 (19)
Paresthesia	2 (17)	0	2 (12)
SARS-CoV-2 infection	2 (17)	0	2 (12)
Treatment-related adverse event†	6 (50)	3 (75)	9 (56)
Serious adverse event	4 (33)	0	4 (25)
Adverse event leading to drug discontinuation, dose interruptions, or trial withdrawal	0	0	0
Adverse event causing death	0	0	0

* Shown are adverse events that emerged or worsened after the first administration of fazirsiran through the end of the trial or early termination. SARS-CoV-2 denotes severe acute respiratory syndrome coronavirus 2.
† The relatedness of adverse events to treatment was determined by the investigator.

RESULTS

PATIENT CHARACTERISTICS

A total of 18 patients were screened, and 16 were enrolled (Fig. S2). The first patient was enrolled on December 19, 2019, and the last patient was enrolled on October 28, 2020. All the patients completed the primary treatment period. Fifteen of 16 patients opted to continue in the extension period. All the patients had received all planned doses by the data-cutoff date (December 6, 2021). Data are presented for the time at which the last patient reached week 48 or 52.

Fourteen patients were evaluable for changes in liver Z-AAT concentration at week 24 or 48, and 15 patients could be assessed for fibrosis. Two patients (in cohort 2) had insufficient liver samples at week 48 for liquid chromatography–tandem mass spectrometry, and 1 patient (cohort 1b) had an insufficient sample for assessment of fibrosis at screening. All the patients were evaluable for PAS-D globule burden and other histologic assessments.

Before trial enrollment, 15 of 16 patients had repeated elevations in liver enzyme concentrations associated with liver disease associated with AAT deficiency. Although F1 to F3 fibrosis (on the basis of a local pathological reading) was an inclusion criterion, 2 patients in cohort 1 had cirrhosis (F4) at screening, and 1 patient in cohort 1b had no fibrosis (F0) (Table 1). Six patients entered the trial while receiving AAT augmentation therapy.

Table 3. Effect of Fazirsiran on Pulmonary Function.*

Variable	Fazirsiran, 100 mg (N=4)	Fazirsiran, 200 mg (N=12)	Total (N=16)
FEV_1 — liters			
Baseline			
No. of patients	4	12	16
Mean	3.28±0.87	3.04±1.08	3.10±1.01
Median	3.16	2.85	2.85
Range	2.48–4.31	1.59–5.30	1.59–5.30
Week 4			
No. of patients	4	12	16
Mean	3.31±0.95	3.01±1.11	3.08±1.05
Median	3.12	2.78	2.81
Range	2.53–4.49	1.62–5.55	1.62–5.55
Week 16			
No. of patients	4	12	16
Mean	3.21±1.01	3.04±1.14	3.08±1.08
Median	3.1	2.81	2.81
Range	2.33–4.30	1.59–5.58	1.59–5.58
Week 28			
No. of patients	4	12	16
Mean	3.26±0.91	3.03±1.09	3.09±1.02
Median	3.15	2.80	2.84
Range	2.44–4.30	1.61–5.31	1.61–5.31
Week 40			
No. of patients	4	12	16
Mean	3.14±1.02	3.01±1.09	3.04±1.04
Median	3.00	2.77	2.77
Range	2.23–4.31	1.64–5.11	1.64–5.11
Week 52			
No. of patients	4	11	15
Mean	3.10±0.80	2.93±1.12	2.98±1.02
Median	3.07	2.89	2.89
Range	2.38–3.88	1.67–5.09	1.67–5.09
$DLCO_{hbg}$ — ml/min/mm Hg			
Baseline			
No. of patients	4	12	16
Mean	22.52±11.40	18.21±8.49	19.28±9.09
Median	18.26	17.23	17.82
Range	14.67–38.89	6.48–35.04	6.48–38.89
Week 4			
No. of patients	4	12	16
Mean	20.73±10.39	18.53±8.01	19.08±8.34
Median	16.92	17.59	16.92
Range	13.23–35.86	8.29–34.33	8.29–35.86

Table 3. (Continued.)

Variable	Fazirsiran, 100 mg (N=4)	Fazirsiran, 200 mg (N=12)	Total (N=16)
Week 16			
No. of patients	2	11	13
Mean	17.22±6.69	19.49±7.39	19.14±7.07
Median	17.22	20.50	20.50
Range	12.49–21.95	10.31–30.91	10.31–30.91
Week 28			
No. of patients	4	11	15
Mean	19.50±10.03	19.66±9.62	19.61±9.37
Median	16.01	18.12	18.12
Range	12.19–33.79	6.66–34.41	6.66–34.41
Week 40			
No. of patients	4	12	16
Mean	19.90±9.20	19.21±8.48	19.38±8.35
Median	16.97	18.04	16.97
Range	12.57–33.08	9.03–34.58	9.03–34.58
Week 52			
No. of patients	4	10	14
Mean	20.98±11.78	18.90±8.22	19.49±8.93
Median	16.37	20.31	19.00
Range	13.10–38.09	7.12–30.56	7.12–38.09

* Plus–minus values are means ±SD. $DLCO_{hbg}$ denotes diffusing capacity of the lungs for carbon monoxide adjusted for hemoglobin concentration.

EFFICACY

Primary End Point

All the patients had reductions in accumulated total liver Z-AAT (median percentage change at week 24 or 48, −83.3%; 95% confidence interval [CI], −89.7 to −76.4) (Fig. 1). Reductions in liver Z-AAT concentrations were similar in the soluble and insoluble fractions (Table S2).

Secondary End Points

A pharmacodynamic response occurred in all the patients after treatment, with a substantial mean (±SD) reduction from baseline in serum Z-AAT concentration in all cohorts. A nadir of −90±5% in the 200-mg cohort and −87±6% in the 100-mg cohort was observed at week 6. The 200-mg cohort had slightly greater sustained reductions in serum Z-AAT concentration than the 100-mg cohort over the 52-week period (Fig. 2A and 2B).

Most patients had a high histologic PAS-D globule burden at baseline (mean score, 7.4; scores range from 0 to 9, with higher scores indicating a greater globule burden) (Fig. S3). After treatment, all the patients had a decreased globule burden, with the mean score decreasing to 2.3 at week 24 or 48 (69% reduction). Figure S4 shows the presence of intrahepatic Z-AAT globules at baseline and fazirsiran-mediated clearance of the globules at week 48.

Reductions in liver Z-AAT concentrations were associated with histologic improvements (reductions) in inflammation. Of the 13 patients with a baseline score of 1 or more (scores range from 0 to 3 [or from 0 to 2 for hepatocyte cell death], with higher scores indicating higher disease activity) who could be evaluated for improvement, approximately two thirds had an improvement of 1 or more points at week 24 or 48 in portal inflammation (8 of 13), interface hepatitis (9 of

13), and hepatocyte cell death (8 of 12), whereas 23% of the patients (3 of 13) had improvements in measures of lobular inflammation by one or more points (Table S3). Meanwhile, 31% of the patients (5 of 16) had worsening in measures of lobular inflammation by 1 or more points.

Biomarkers of liver injury were also reduced. At baseline, mean ALT concentrations were above the upper limit of the normal range in all cohorts. After treatment, ALT concentrations decreased in all cohorts from week 16 through week 52 (Fig. 2C). All 12 patients with ALT concentrations above the upper limit of the normal range at baseline had reductions to normal concentrations at week 52. Mean AST concentrations were similarly reduced (data not shown). Mean γ-glutamyltransferase concentrations also decreased after treatment (Fig. 2D). Four of 8 patients (50%) with baseline γ-glutamyltransferase concentrations above the upper limit of the normal range had normal concentrations at week 52.

Regression of fibrosis (≥1 stage) occurred in 7 of 12 patients receiving the 200-mg dose (cohorts 1 and 2), including the 2 patients with cirrhosis, and in none of 3 patients with evaluable biopsies who received the 100-mg dose (cohort 1b) (Fig. S5). Two patients in cohort 2 had progression of fibrosis from baseline to week 48 (both from F2 to F3), although both had profound reductions in PAS-D globule burden (scores of 9 and 4 at baseline and 0 for both at week 48) and reduced ALT and γ-glutamyltransferase concentrations with treatment.

Mean concentrations of serum Pro-C3 decreased in cohorts 1 and 2 but remained relatively unchanged from baseline in cohort 1b through the last observation at week 28. Serum Pro-C3 concentrations decreased by 36% in cohort 1 at week 28 and remained low through week 52 (when the percentage by which the concentration had decreased was 39%). A sustained but less pronounced decrease was observed in cohort 2 (Fig. S6).

Exploratory End Point

Pre- and postdose assessments of liver stiffness are suggestive of moderate reductions in cohorts 1 and 2 and show no change in cohort 1b. The mean (±SD) percentage change from baseline was –18±11% at week 24 in cohort 1, –15±35% at week 48 in cohort 2, and –2±16% in cohort 1b. Among all the patients, the change in liver stiffness was –12% from baseline at week 24 or 48 (Table S5).

SAFETY

Over a period of 1.5 years, there were no deaths, discontinuations of treatment with fazirsiran, or dose interruptions (Table 2). The most common adverse events that emerged or worsened after the first administration of fazirsiran were arthralgia and increased concentrations of blood creatinine kinase (four patients [25%] each). Adverse events involving increased blood creatinine kinase concentrations were mild and transient. There were no apparent dose-dependent increases in the frequency or severity of adverse events. Four serious adverse events, all moderate in severity, were reported in cohorts 1 and 2: viral myocarditis, diverticulitis, dyspnea, and vestibular neuronitis. The case of myocarditis was associated with Epstein–Barr virus infection. The case of dyspnea occurred in a patient with nonobstructive pulmonary emphysema and delayed pulmonary care, with no substantial changes in lung function during the trial. The case of vestibular neuronitis occurred after vaccination against coronavirus disease 2019 (Covid-19). Each serious event resolved, and each of the four patients continues to receive fazirsiran treatment in the extension period.

Patients with the PI ZZ genotype are severely deficient in serum AAT protein and that which is present is poorly functional. Because fazirsiran silences expression of AAT, there is a risk that depletion of already low concentrations of circulating Z-AAT may impair pulmonary function or exacerbate preexisting emphysema. So far, there have been no major pulmonary adverse events resulting in drug or trial discontinuations. Four of the six patients who entered the trial while receiving AAT augmentation therapy had a history of emphysema, and none reported exacerbations. Locally conducted pulmonary-function tests showed fluctuations in FEV_1 (Table 3). Overall, mean FEV_1 values were 3.1 liters at baseline, 3.1 liters at week 28, and 3.0 liters at week 52. The median FEV_1 remained stable over time: 2.85 liters at baseline, 2.84 liters at week 28, and 2.89 liters at week 52. The mean $DLCO_{hbg}$ was also stable over time, ranging from 19.1 to 19.6 ml per minute per mm Hg (Table 3).

DISCUSSION

Liver injury in liver disease associated with AAT deficiency results from the accumulation of the Z-AAT protein in hepatocytes.[1,5,13,14] Preclinical evidence suggests that Z-AAT accumulation in hepatocytes is counteracted with multiple intracellular pathways (e.g., proteasome degradation and autophagy) in an attempt to reduce the injurious consequences of the accumulation.[5,10,13,15-17] Patients with other chronic liver diseases, such as nonalcoholic fatty liver disease, undergo similar hepatocellular changes in response to the accumulation of the insult (e.g., excess fat), which can progressively lead to inflammation, tissue regeneration, and fibrogenesis.[18] Through an RNAi mechanism, fazirsiran treatment reduced new Z-AAT synthesis, allowing these pathways to clear the toxic Z-AAT accumulation and thereby removing the insult and allowing native liver-restorative processes to function.

Z-AAT accumulation in the liver correlates with advanced liver fibrosis,[9] and progression of fibrosis is often accompanied by elevations in liver enzyme concentrations and portal inflammation.[13,14] Because the liver is a regenerative organ, removal of the Z-AAT hepatic insult is expected to yield clinical benefit. This concept has been demonstrated in the treatment of other chronic liver diseases (e.g., hepatitis B and C) in which removal of the etiologic factor prevents progression of fibrosis and may reverse established fibrosis and even cirrhosis.[19] Improvements in fibrotic-tissue architecture are likely to prevent hepatic decompensation and reduce liver-related morbidity and mortality.[20,21]

Most end points in this trial were objective laboratory or imaging variables. Changes in histologic measures were assessed and adjudicated by three independent hepatopathologists who were unaware of the cohort assignments. All the patients had marked reductions in total liver Z-AAT concentrations and PAS-D globule burden over a period of 24 or 48 weeks. Changes in liver Z-AAT concentrations appeared to be moderately associated with changes in portal inflammation and liver enzyme concentrations. However, establishing a correlation between liver Z-AAT concentrations and these other biomarkers is not possible in this trial, given the small number of patients and the consistent association between receipt of fazirsiran and lower concentrations of liver Z-AAT. We observed a change in the stage of fibrosis in a subgroup of patients, the degree of which was independent of the magnitude of the reduction in liver Z-AAT concentrations. Nevertheless, of the 12 patients who received the 200-mg dose, 7 had regression of fibrosis by one or more stages after 24 or 48 weeks. Two patients showed progression of fibrosis by one stage at week 48, and yet we observed in these same patients profound reductions in globule burden and liver enzyme concentrations. The apparent increase in fibrosis in these 2 patients may reflect variability due to sampling error or interreader variability.

In this trial, a gradual decrease in mean FEV_1 was observed through week 52, although the median FEV_1 and mean $DLCO_{hbg}$ remained stable. There was no clear evidence to suggest that observed changes in pulmonary-function measurements were associated with fazirsiran treatment. These data have some limitations owing to the small sample, lack of control group, and fluctuations in FEV_1 across trial visits. The causes of changes in pulmonary-function measurements almost certainly include factors such as preexisting respiratory medical conditions, intercurrent events that occurred before observed decreases in pulmonary function (e.g., infection or Covid-19), and natural disease progression in patients with lung disease associated with AAT deficiency. The fluctuations are probably due, in part, to the fact that pulmonary-function tests were conducted at sites without centralized interpretation or quality assessment, a known issue with FEV_1 assessments. FEV_1 can vary by up to 20% in patients with chronic obstructive pulmonary disease without central monitoring, and central monitoring improves the precision of sequential measurements.[22,23] So far, however, the decrease in serum Z-AAT concentrations after fazirsiran therapy has not been accompanied by exacerbation of pulmonary disease in this small group of patients, even among those with preexisting emphysema. Long-term controlled studies with a larger sample and centralized monitoring will be needed to evaluate pulmonary function and determine whether the decrease differs from that in an untreated group.

RNAi therapeutics represent a growing class of approved or investigational drugs that have

the potential to silence specific genes in targeted cell types. Advancements in liver-targeted RNAi therapeutics for metabolic disorders such as hyperlipidemias or diseases originating in the liver (e.g., primary hyperoxaluria) represent breakthroughs.[24] This trial shows that fazirsiran reduced production and accumulation of intrahepatic Z-AAT, the causal factor in liver disease associated with AAT deficiency, and was associated with concurrent improvements in biochemical and histologic biomarkers of inflammation. Despite marked reductions in liver Z-AAT concentrations in all the patients, reductions in mutant protein concentrations did not uniformly translate into regression of fibrosis during the first 24 or 48 weeks of treatment. Ultimately, the final goal and clinical benefit of treatment for patients with liver disease associated with AAT deficiency will be the prevention or regression of fibrosis. Therefore, placebo-controlled clinical trials with larger samples and longer treatment duration will be needed to confirm the effect of fazirsiran on fibrosis.

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