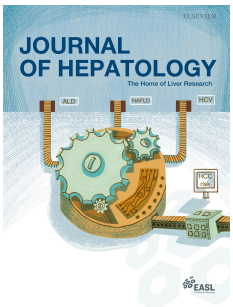

A phase I/II study of ARO-HSD, an RNA interference therapeutic, for the treatment of non-alcoholic steatohepatitis

Lung-Yi Mak, Ed Gane, Christian Schwabe, Ki Tae Yoon, Jeong Heo, Russell Scott, Jeong-Hoon Lee, Jung Il Lee, Young Oh Kweon, Martin Weltman, Stephen A. Harrison, Brent A. Neuschwander-Tetri, Kenneth Cusi, Rohit Loomba, Bruce D. Given, Dawn R. Christianson, Eric Garcia-Medel, Min Yi, James Hamilton, Man-Fung Yuen

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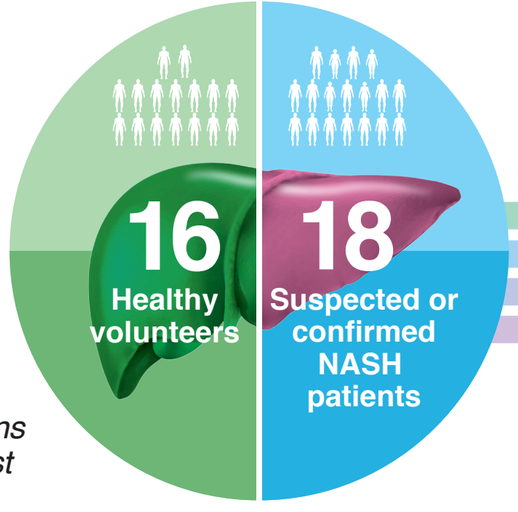
Can a hepatocyte targeted siRNA therapeutic mimic a genetic mutation that protects against alcoholic and non-alcoholic liver disease including NASH?

ARO-HSD is a hepatocyte targeted siRNA therapeutic designed to silence HSD17β13 expression

ARO-HSD

HSD17β13 loss-of-function mutations have been shown to protect against alcoholic and non-alcoholic liver disease including NASH

Study Cohorts



Results Observed

Favorable safety profile
ARO-HSD was well tolerated with no treatment-related serious adverse events or drug discontinuations

Hepatic *HSD17β13* mRNA reduction –94.4%
Reduction from baseline at day 71 at 200mg

Hepatic HSD17β13 protein reduction –83.0%
Reduction from baseline at day 71 at 200mg

ALT reduction –42.3%
Mean change from baseline at day 71 at 200mg

Proof of Concept

In this proof-of-concept study, ARO-HSD was well tolerated and able to significantly reduce HSD17β13 expression in a group of 18 patients, resulting in an improvement in markers of liver injury

Lung-Yi Mak, Ed Gane, Christian Schwabe, Ki Tae Yoon, Jeong Heo, Russell Scott, Jeong-Hoon Lee, Jung Il Lee, Young Oh Kweon, Martin Weltman, Stephen A. Harrison, Brent A. Neuschwander-Tetri, Kenneth Cusi, Rohit Loomba, Bruce D. Given, Dawn R. Christianson, Eric Garcia-Medel, Min Yi, James Hamilton, Man Fung Yuen. 2022

TITLE PAGE

Title:

A phase I/II study of ARO-HSD, an RNA interference therapeutic, for the treatment of non-alcoholic steatohepatitis

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Trial Registration Number	ClinicalTrials.gov number, NCT04202354
Word Count	Abstract: 274 Manuscript (including abstract, references, tables, and figure legends): 5523

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	Axcella Health, Boston Pharmaceuticals, B Riley FBR, BVF Partners, Cohbar, Canfite, Corcept Therapeutics, Cymabay Therapeutics, Echosens North America, Enyo Pharma SA, Fibronostics, Foresite Labs, Fortress Biotech, Galectin Therapeutics, Genfit Corp, GNS, Hepion Pharmaceuticals, HighTide Therapeutics, HistoIndex PTE, Inipharm, Intercept Pharmaceuticals, Ionis, Kowa Research Institute, Madrigal Pharmaceuticals, Medpace, Metacrine, Microbe, NGM Biopharmaceuticals, Northsea Therapeutics BV, Novo Nordisk, Nutrasource, Perspectum Diagnostics, Piper Sandler, Poxel, Prometic, Pharma SMT, Ridgeline, Sagimet Biosciences, Sonic Incytes Medical, Terns, and Viking Therapeutics; is on an advisory board or panel for 89 Bio, Akero Therapeutics, Altimmune, Arrowhead Pharmaceuticals, Axcella Health, Chronwell, CiVi, Cymabay Therapeutics, Echosens North America, Foresite Labs, Galectin Therapeutics, Galmed Research & Development, Genfit, Gilead Sciences, Hepion Pharmaceuticals, HighTide Therapeutics, HistoIndex PTE, Indalo, Intercept Pharmaceuticals, Madrigal Pharmaceuticals, Medpace, Metacrine, NGM Biopharmaceuticals, Northsea Therapeutics BV, Novartis Pharmaceutical, Novo Nordisk, PathAI, Poxel, Prometic Pharma SMT, Ridgeline, Sagimet Biosciences, Sonic Incytes Medical, Terns, and Theratechnologies; and has stock/shares for Akero Therapeutics, Chronwell, Cirius Therapeutics, Galectin Therapeutics, Genfit, Hepion Pharmaceutical, HistoIndex PTE, Metacrine, NGM Biopharmaceuticals, Northsea Therapeutics BV, and

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	Pharmaceuticals, AstraZeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Eli Lilly, Galectin Therapeutics, Galmed Pharmaceuticals, Gilead, Hanmi, Intercept, Inventiva, Ionis, Janssen, Madrigal Pharmaceuticals, Merck, NGM Biopharmaceuticals, Novo Nordisk, Pfizer, Sonic Incytes, and Terns Pharmaceuticals; and he co-founded LipoNexus Inc. BG, DC, EGM, MY, JSM, and JHamilton are/were employees of Arrowhead and are Arrowhead shareholders. BG receives consulting fees from Arrowhead Pharmaceuticals. MFY has participated on a Data Safety Monitoring Board or Advisory Board for AbbVie, Aligos Therapeutics, AiCuris, Antios Therapeutics, Assembly Biosciences, Arbutus Biopharma, Bluejay Therapeutics, Clear B Therapeutics, Dicerna Pharmaceuticals, Finch Therapeutics, GlaxoSmithKline, Gilead Sciences, Janssen, Merck Sharp and Dohme, Hoffmann-La Roche, and Vir Biotechnology.
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Author Contribution Statement	Arrowhead Pharmaceuticals designed and conducted the study and collected and analyzed the data. All authors interpreted the data and reviewed and collaborated in the preparation of the manuscript. The first author wrote the first and all revisions of the manuscript with assistance from coauthors from Arrowhead. All authors reviewed and approved the final version, made the decision to submit the manuscript for publication,

	and vouched for the accuracy and completeness of the data and for the fidelity of the study to the protocol.

ABSTRACT

Background:

Loss-of-function HSD17β13 mutations are protective against development of chronic liver disease. HSD17β13 inhibition represents a potential approach to treat liver disease such as non-alcoholic steatohepatitis (NASH). ARO-HSD is an RNA interference (RNAi) therapeutic designed to selectively reduce expression of *HSD17β13* messenger RNA (mRNA) in hepatocytes. The study evaluated the effects of ARO-HSD in normal healthy volunteers (NHVs) and patients with confirmed or clinically suspected NASH.

Methods:

This dose-escalating study evaluated the safety, tolerability, and pharmacodynamics of ARO-HSD in 32 NHVs and 18 patients with confirmed/clinically suspected NASH.

Double-blind NHV cohorts received single escalating doses of ARO-HSD (25, 50, 100, or 200 mg) or placebo subcutaneously on Day 1. Open-label patient cohorts received ARO-HSD (25, 100, or 200 mg) subcutaneously on Days 1 and 29. Liver biopsy was performed in patients predose and Day 71 to evaluate expression levels of *HSD17β13* mRNA and protein.

Results:

ARO-HSD treatment was well tolerated with no treatment-related serious adverse events or drug discontinuations. The most frequently reported treatment-emergent adverse events were mild injection site reactions short in duration. Mean changes from baseline at Day 71 in hepatic *HSD17β13* mRNA were -56.9% (25 mg), -85.5% (100 mg), and -93.4% (200 mg). The overall pooled subject *HSD17β13* mRNA reduction was 78.6% ($p<0.0001$). Hepatic HSD17β13 protein levels were similarly reduced across doses. In patients, mean changes in alanine

aminotransferase (ALT) from baseline at Day 71 were -7.7% (25 mg), -39.3% (100 mg), and -42.3% (200 mg) ($p<0.001$ for pooled cohorts).

Conclusions:

ARO-HSD was well tolerated at doses ≤200 mg. This proof-of-concept study demonstrated that short-term treatment with ARO-HSD reduces hepatic *HSD17β13* mRNA and protein, which is accompanied by reductions in ALT.

Impacts and Implications:

- There is an unmet medical need for new therapies to treat alcoholic and nonalcoholic liver disease.
- ARO-HSD is an siRNA therapy, designed to silence HSD17β13 expression with an intent to phenocopy the protective effect seen in both alcoholic and nonalcoholic liver disease with HSD17β13 loss-of-function.
- The reductions in HSD17β13 expression and in transaminases seen with ARO-HSD administration represent an initial step towards clinical validation of HSD17β13, a drug target with substantial genetic validation, as an important modulator of human liver disease.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease with a worldwide prevalence of 20% to 30%.[1] Non-alcoholic steatohepatitis (NASH), which can currently only be confirmed with liver biopsy, is the progressive form of NAFLD in which hepatocyte injury and inflammation have developed in the setting of background steatosis. NAFLD is strongly associated with metabolic syndrome, type 2 diabetes mellitus, and obesity, with up to 20% to 30% of NAFLD patients developing NASH.[1,2,3] Development of NASH depends on multiple factors and may be influenced by the presence of several genetic variants.[4,5] Driven by rising obesity, prevalence estimates for NASH are as high as 5% of the global and US population.[3,6] Prevalence rates for NASH in the middle-aged population in the US are estimated to range from 5% to 14%.[7] Patients with NASH are at increased risk of both cardiovascular- and liver-related adverse outcomes, with NASH currently the second leading cause of liver transplantation in the US.[8]

Although lifestyle modification, including diet and exercise, can be an effective intervention in NASH, long-term adherence is poor, and it may not be sufficient to resolve disease in all patients, particularly those with advanced disease. There are no drugs approved by the US Food and Drug Administration (FDA) or European Medicines Agency (EMA) to treat NASH and there exists an unmet need for NASH therapeutic options.

HSD17β13 is a hydroxysteroid dehydrogenase possibly involved in the metabolism of hormones, fatty acids, and bile acids. In humans, it is abundantly expressed in the liver, localizing to the surface of lipid droplets as an active dehydrogenase against substrates such as retinol[9] and estradiol,[10] but with physiologically relevant substrates still unknown. Hepatic *HSD17β13* expression appears limited to hepatocytes.[9] Human genetic studies indicate that loss-of-function

(LOF) mutations in *HSD17β13* are protective against development of both alcohol-related and non-alcohol-related liver disease, with approximately 30% to 50% risk reductions compared to noncarriers associated with the LOF splice variant rs72613567.[10] This protective effect seen in individuals with LOF mutations may indicate a potential role for inhibition of *HSD17β13* in the treatment of liver diseases such as NASH.

ARO-HSD is an RNA interference (RNAi)-based therapeutic composed of a synthetic double-stranded oligonucleotide designed to selectively target *HSD17β13* messenger RNA (mRNA) in hepatocytes. To promote liver specificity, ARO-HSD was conjugated to galactose-containing ligands that are preferentially taken up by hepatocytes. Once in the cytoplasm, ARO-HSD engages the cell's RNA-induced silencing complex (RISC) to target *HSD17β13* mRNA for degradation. These factors likely minimize any potential extrahepatic inhibition of *HSD17β13* expression. Treatment with ARO-HSD is expected to reduce hepatocyte production of the HSD17β13 protein. Based on human genetic data, this approach may have a protective and/or therapeutic effect in patients with NASH. The clinical study evaluated the safety, tolerability, pharmacokinetic, and pharmacodynamic effects of ARO-HSD in NHVs and patients with confirmed/clinically suspected NASH.

METHODS

Participants and Study Design

This was a multicenter, phase 1/2a clinical study (Clinicaltrials.gov NCT04202354) in NHVs and patients with confirmed NASH (by prior biopsy) or clinically suspected NASH. A historical liver biopsy read locally as demonstrating NASH within 1 year of the Screening visit was considered confirmed NASH. Histologic evaluation was not completed (either predose or

postdose) as part of this clinical study. Clinically suspected NASH was based on magnetic resonance imaging proton density fat fraction (MRI-PDFF) >8% with ALT >upper limit of normal (30 U/L for men and 19 U/L for women).[11]

The study was conducted in adult males and females, ages 18 through 55 years, with body mass index (BMI) <35 kg/m² (NHVs) or ages 19 through 65 years with BMI ≤40.0 kg/m² (patients with confirmed/clinically suspected NASH).

Genotyping was performed at Screening in patients with confirmed/clinically suspected NASH for *HSD17β13* rs72613567 and patatin-like phospholipase domain containing 3 (*PNPLA3*) rs738409 (I148M) variants. Individuals were categorized by *HSD17β13* rs726131567 genotypes into T/T (homozygous wild-type, or no mutation) or T/TA (heterozygous mutation) groups. Individuals who were homozygotes for rs726131567:TA were excluded from study. Similarly, individuals were categorized by *PNPLA3* genotypes into C/C (homozygous wild-type, or no mutation), C/G (heterozygous mutation), or G/G (homozygous mutation) groups.

Subjects enrolled into the study in a double-blind (NHV Cohorts 1 [25 mg], 2 [50 mg], 3 [100 mg], and 4 [200 mg]) or open-label (patient Cohorts 1b [25 mg], 3b [100 mg], and 4b [200 mg]) fashion (Supplementary Table 1, Supplementary Figure 2). NHV subjects were randomized at a ratio of 1:1 (active:placebo) to receive a single subcutaneous (SC) injection of either ARO-HSD or placebo (Cohorts 1 through 4) on Day 1. Patients with confirmed/clinically suspected NASH received open-label ARO-HSD (Cohorts 1b, 3b, and 4b) on Days 1 and 29. These patients were not randomized into any specific cohort. Patient Cohorts 3b and 4b were enrolled sequentially, and Cohort 1b was added later (to better evaluate dose response) and was enrolled after Cohorts 3b and 4b were fully enrolled. The study design is summarized in Supplementary Figure 1 and Supplementary Table 1.

Study Treatments

Fifty subjects (32 NHVs and 18 patients) were enrolled at study centers in Australia, New Zealand, Hong Kong, and South Korea. Of the 58 NHVs screened, 8 were screen failed (mostly due to not meeting inclusion or exclusion criteria) and 18 were not assigned (due to a lapse in 45-day Screening Period because of the coronavirus disease 2019 [COVID-19] global pandemic) (Supplementary Figure 2A). For NHV Cohorts 1 through 4, each double-blind cohort enrolled 8 subjects (4 active:4 placebo) (Supplementary Figure 2A). NHV cohorts received single doses of ARO-HSD or placebo at dose levels 25 mg (Cohort 1), 50 mg (Cohort 2), 100 mg (Cohort 3), and 200 mg (Cohort 4) on Day 1.

Among the 34 patients with either NASH (based on liver biopsy within 1 year prior to enrollment) or clinically suspected NASH that were screened, 16 were screen failed (due to not meeting inclusion or exclusion criteria) (Supplementary Table 1, Supplementary Table 3, Supplementary Figure 2B). For the 18 patients entering the open-label Cohorts 1b, 3b, and 4b, 6 were enrolled per cohort. All patient cohorts received ARO-HSD at dose levels 25 mg (Cohort 1b), 100 mg (Cohort 3b), or 200 mg (Cohort 4b) on Days 1 and 29. Patients were followed until Day 113 (Week 16).

Study Assessments and Procedures

In patients with confirmed/clinically suspected NASH, pharmacodynamic effects of ARO-HSD were measured by hepatic *HSD17β13* mRNA (quantitative reverse transcription-polymerase chain reaction [qRT-PCR]) and protein (Western Blot) expression, taken from paired needle biopsies of the liver at Screening and Day 71. For gene expression measurements by qRT-PCR, *HSD17β13* and beta-actin (endogenous control) mRNA were measured at both Screening and Day 71. Relative expression was calculated using the $2^{-\Delta\Delta C_T}$ method of normalization.[12] Changes

to HSD17β13 protein expression were assessed as the ratios of HSD17β13/vinculin protein at Day 71 relative to predose baseline levels. Detailed information describing the Western Blot by ProteinSimple capillary electrophoresis and protein quantitation is provided in the Supplementary Material and Methods. Markers of liver injury, such as ALT and aspartate aminotransferase (AST), were measured from Screening to Day 113. Liver imaging was performed to assess changes in liver fat fraction using MRI-PDFF, and liver stiffness using transient elastography (FibroScan®) at Screening and Day 71. *HSD17β13* rs72613567 and *PNPLA3* rs738409 single nucleotide polymorphisms were detected in whole blood by real-time PCR, and results were confirmed using Sanger sequencing at Screening (see Supplementary Materials and Methods).

Other exploratory or NASH-related biomarkers were measured from Screening to Day 113. A full list of assessments is provided in the clinical study protocol. However, some planned biomarkers were not measured due to assay feasibility. Results, including percent change from baseline and duration of response (when applicable), were analyzed and summarized by dose cohort.

Safety assessments included incidence of adverse events (AEs)/serious AEs (SAEs), physical examinations, and vital sign measurements (blood pressure, heart rate, temperature, and respiratory rate), electrocardiogram (ECG), clinical laboratory tests (blood and urine), concomitant medications/therapy, and reasons for treatment discontinuation in both NHV and patient cohorts.

Statistical Analysis

No formal sample size calculation was conducted for this first-in-human clinical study.

All safety results, demographic and baseline characteristics data, and pharmacodynamic results were summarized based on data collected from all enrolled subjects who received at least one dose of active drug or placebo. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA, Version 24.0).

For analyses of pharmacodynamic parameters, descriptive statistics summaries and derived knockdown data from baseline were provided for *HSD17β13* mRNA and protein. A non-parametric Wilcoxon signed-rank test was performed to test the significance of change from baseline at Day 71 for *HSD17β13* mRNA and protein. For liver enzyme parameters such as ALT and AST, descriptive statistics summaries were provided along with derived change from baseline and percent change from baseline summaries. A non-parametric Wilcoxon signed-rank test was performed to test the significance of change from baseline at Days 29, 71, and 113 for ALT and AST. For hepatic imaging (MRI-PDFF and FibroScan), descriptive statistics summaries and derived percent change from baseline were provided. Baseline values were defined as the last non-missing observation (predose value closest to the first dose) for each subject prior to the dosing of study medication (ie, start of injection on Day 1). The data were analyzed using SAS software, Version 9.4 (SAS Institute, Inc., Cary, NC, USA), and figures were generated using GraphPad Prism software, Version 8.3.0 (GraphPad Software, San Diego, CA, USA).

Study Oversight

The ethics committee at each participating center approved the protocol, and the study was conducted in accordance with the principles of the Declaration of Helsinki, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

(ICH) Good Clinical Practice guidelines, and applicable regulatory requirements. All subjects provided written informed consent before enrollment.

RESULTS

Patient Characteristics and Disposition

Healthy volunteers:

The demographic characteristics for NHVs are described in the supplemental information (Supplementary Table 2). All enrolled NHVs completed all planned assessments.

Patients with confirmed or clinically suspected NASH:

The demographic characteristics for patients are described in Table 1. Of the 18 patients who enrolled in the study, 4 patients had confirmed NASH (based on prior biopsy). The mean (SD) age was 44.5 (9.9) years. Four subjects (22.2%) were female and 14 subjects (77.8%) were male. Thirteen subjects (72.2%) were Asian, 3 subjects (16.7%) were White, 1 subject (5.6%) was Black or African American, and 1 subject (5.6%) was other. No patients had Hispanic or Latino ethnicity. At baseline, the mean MRI-PDFF was 19.9% (range 10.3%-30.7%) (Supplementary Table 4). The mean ALT at baseline was 63.3 U/L (range 28 U/L [which was for a female subject] to 144 U/L) (Table 1).

These patients presented with typical NAFLD comorbidities, with 50.0% (n=9), 44.4% (n=8), and 44.4% (n=8) having hyperlipidemia, type 2 diabetes mellitus, and hypertension, respectively.

Baseline concomitant medications taken by patients with confirmed/clinically suspected NASH included statins (HMG-CoA reductase inhibitors) (7 subjects [38.9%]), biguanides (metformin) (6 subjects [33.3%]), one or more anti-hypertensive (14 subjects [77.8%]), glucagon-like

peptide-1 (GLP-1) analogues (1 subject [5.6%]), and sodium-glucose cotransporter 2 (SGLT2) inhibitors (1 subject [5.6%]).

A summary of genotyping results for patients by cohort is provided in Table 1. For the *HSD17β13* rs72613567 variant, 12 subjects (66.7%) had no mutation detected (T/T), and 6 subjects (33.3%) were heterozygotes (T/TA). For the *PNPLA3* rs738409 variant, 11 subjects (61.1%) had no mutation detected (C/C), 4 subjects (22.2%) were heterozygotes (C/G), and 3 subjects (16.7%) were homozygotes (G/G).

All subjects completed the study without early termination.

Pharmacodynamics

Hepatic HSD17β13 mRNA and Protein

ARO-HSD reduced the hepatic mRNA encoding HSD17β13 protein from the pretreatment baseline by a mean (min–max) value of 56.9% (50.7%-60.5%), 85.5% (61.6%-96.1%), and 93.4% (90.8%-98.6%) in the 25, 100, and 200 mg dose cohorts, respectively, on Day 71 (Figure 1). The overall pooled subject *HSD17β13* mRNA reduction was 78.6% (50.7%-98.6%, $p<0.0001$). In the 200 mg dose cohort, all 6 subjects demonstrated >90% reduction in hepatic expression of *HSD17β13* mRNA. Hepatic HSD17β13 protein levels were similarly reduced by a mean of >33.8%, >86.0%, and 83.0% in the 25, 100, and 200 mg dose cohorts, respectively, and average change from baseline in HSD17β13 protein from pooled cohorts was -63.2% (-97.8%-53.8%, $p=0.0017$), with multiple measurements (for 3 patients at baseline and 6 patients on Day 71) that were below the assay's level of quantitation (Table 3). Profiles for different biomarkers (*HSD17β13* and *PNPLA3* mRNA expression, HSD17β13 protein, FibroScan, MRI-PDFF, ALT, and AST) for patients with confirmed/clinically suspected NASH are presented in

Supplementary Tables 6 and 7. The observed data for *HSD17β13* mRNA reduction suggest that robust on-target pharmacological responses were achieved in the liver with trends toward dose dependency between the 100 and 200 mg dose levels, and clear dose dependency between the 25 and 100 mg dose levels. Observed pharmacodynamic effects were not affected by *HSD17β13* (rs72613567, T>TA) or *PNPLA3* (rs738409, C>G) mutations. No difference was observed in *HSD17β13* mRNA or protein reduction between patients that either had no mutation or were heterozygotes for rs72613567 splice variant (Figure 2A, Supplementary Figure 3). Similarly, there was no difference in *HSD17β13* mRNA reduction between patients that either had no mutation or were heterozygotes or homozygotes for *PNPLA3* rs738409 polymorphism (Figure 2B). Thus, *HSD17β13* knockdown was consistent in this study regardless of genotype or mutation status.

ALT and AST

In patients with confirmed/clinically suspected NASH, serum biomarkers of liver injury decreased following treatment with ARO-HSD, demonstrating dose-dependent reduction in ALT between the 25 and 100 mg dose levels. The 100 and 200 mg dose levels showed similar reductions in ALT whereas no significant reductions were observed at the 25 mg dose level. The mean percent change from baseline at Day 71 (same time as biopsy) for ALT was -7.7% (25 mg), -39.3% (100 mg), and -42.3% (200 mg) ($p<0.001$ for pooled cohorts) (Figure 3A). At Day 113 (end of study), the mean percent change from baseline for ALT was -14.1%, -36.0%, and -39.4% for the 25, 100, and 200 mg dose levels, respectively ($p<0.01$ for pooled cohorts) (Figure 3A). Overall, ALT levels were decreased to <53 U/L (the central laboratory normal limit) following ARO-HSD treatment for 50.0% of patients by end of study.

Similar to ALT, AST was reduced in a dose-dependent manner between the 25 and 100 mg dose levels, with similar reductions observed between the 100 and 200 mg doses. The mean percent change from baseline at Day 71 for AST was 4.1%, -24.5%, and -28.3% for the 25, 100, and 200 mg dose levels, respectively (p=0.016 for pooled cohorts) (Figure 3B). At Day 113, mean percent change from baseline for AST was -7.5%, -14.7%, and -19.8% for the 25, 100, and 200 mg dose levels, respectively (Figure 3B). See Supplementary Table 7 for individual patient data.

Mean body weight was stable for the duration of the study within each dose group. Two subjects (25 mg and 100 mg groups) lost weight during the study and showed the largest percent decrease in ALT from baseline within their dose groups. These subjects had decreases in body weight of 4.6 kg (25 mg group) and 2.2 kg (100 mg group) at Day 71 corresponding to 60% and 51% reductions in ALT, respectively. Most other subjects maintained stable body weight throughout the study; therefore, all other decreases in liver enzymes were observed independent of changes in body weight.

Lipids and Metabolic Markers

A summary of change from baseline in lipid parameters and metabolic markers (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, glucose, hemoglobin A1c, hemoglobin, and platelets) for patients with confirmed/clinically suspected NASH is provided in Supplementary Table 5. There were no clinically meaningful effects on lipids or other metabolic parameters following ARO-HSD administration at all dose levels.

Liver Fat Fraction and Liver Stiffness

The mean relative changes from baseline at Day 71 in liver fat fraction by MRI-PDFF were 14.4%, -7.6%, and -7.3% at doses 25, 100, and 200 mg, respectively (Supplementary Figure 5A, Supplementary Table 4). Individual changes in liver fat fraction within each dose group were highly variable, ranging from -36.0% to 87.9% (25 mg), -40.7% to 23.6% (100 mg), and -24.1% to 5.9% (200 mg) (Supplementary Figure 5B, Supplementary Table 4). Overall, relative changes from baseline in liver fat fraction were not significantly different (p=0.5 for pooled cohorts). A subject in the 25 mg group that had the notably highest relative increase in liver fat fraction of 87.9% also had the highest weight increase from baseline of 6.9 kg at Day 71 and an overall increase in liver enzymes (ALT, AST, and gamma-glutamyl transferase [GGT]) by end of study.

The mean percent change from baseline for liver stiffness (in kPa) at Day 71 was +16.7%, +2.2%, and +4.2% at doses 25, 100, and 200 mg, respectively (Supplementary Figure 5C, Supplementary Table 4). However, these changes were not statistically significant. Individual changes were highly variable within each cohort, ranging from -5.3% to 37.5% (25 mg), -16.5% to 33.9% (100 mg), and -36.8% to 54.4% (200 mg) (Supplementary Figure 5D, Supplementary Table 7).

Safety

ARO-HSD was well tolerated in both NHVs and patients with confirmed/clinically suspected NASH (Table 2, Supplementary Table 8) with no AE-related study or drug discontinuations, no dose-limiting toxicities, or deaths. There was no recurrent pattern of adverse laboratory findings indicative of end organ toxicity. The most frequently reported treatment-emergent AEs (TEAEs) were injection site reactions (injection site bruising, injection site erythema, and injection site swelling), only seen in NHVs, that were mild in severity and short in duration (Supplementary Table 8). The remaining TEAEs were those commonly observed in clinical studies (Table 2). A

single SAE that required hospitalization was reported for a subject in the 200 mg cohort. The subject was admitted for a right arm soft tissue injury following an accident that occurred at work (unrelated to the time and site of injection) and was discharged from the hospital 2 days later. The subject recovered from the event and continued in the study. The SAE was considered not related to study drug.

DISCUSSION

Hepatic *HSD17β13* expression is markedly upregulated in liver biopsies of humans with NAFLD where the protein is associated with hepatocyte lipid droplets.[13] HSD17β13 is one of the few NASH drug targets supported by human genetic data where LOF variants are associated with reduced risk of NASH.[10,14] Reducing mRNA expression by RNA interference represents a useful method for recapitulating beneficial phenotypes conferred by LOF mutations. In our cohort of patients with confirmed/clinically suspected NASH, treatment with ARO-HSD led to dose-dependent reductions in *HSD17β13* mRNA expression between the 25 and 100 mg dose levels, based on postdose Day 71 biopsies. For the 100 and 200 mg dose levels, there was a trend towards dose dependency with all patients achieving reductions of >90% at the 200 mg dose. These findings clearly demonstrated potent target engagement with knockdown of *HSD17β13*, mimicking a LOF mutation in NASH. Reduced *HSD17β13* expression was accompanied by reductions in ALT, a widely used surrogate marker of hepatic inflammation in NASH.[15,16] While this would need to be confirmed by histology and longer treatment, reductions in ALT have been shown to be associated with improvements in histologic markers of NASH.[15,16] The observed magnitude in ALT decline generally correlated with increased ARO-HSD dose level, as observed between the low dose (25 mg) compared to higher dose levels (100 and 200 mg). This

finding mimics the lower ALTs described in genetic studies of subjects harboring *HSD17β13* LOF variants[10,17] and supports the overall hypothesis of targeting *HSD17β13* with RNAi as a potential treatment of NASH.

The *HSD17β13* LOF variant gene has been shown to be associated with reduced risk of liver disease, even in individuals carrying the *I148M PNPLA3* variant.[10,17] Therefore, *HSD17β13* knockdown could potentially mitigate the severity of liver disease in patients with genetic polymorphisms of *PNPLA3*. In our current study, inhibition of HSD17β13 was induced by ARO-HSD in a dose-dependent manner, and the inhibition was consistent across subjects with the *PNPLA3* rs738409 CG or GG genotypes that are classically associated with increased risk of NASH, cirrhosis, and hepatocellular carcinoma.

While ARO-HSD reduced *HSD17β13* gene expression with associated reductions in ALT, there was no clear reduction in hepatic steatosis as measured by MRI-PDFF. This is not unexpected as several studies have shown that individuals harboring *HSD17β13* LOF variants do not have reduced liver fat relative to controls and in some cases may have increased levels of hepatic steatosis.[9,18] The finding that ARO-HSD does not seem to directly impact hepatic steatosis, albeit in a small number of patients, is consistent with human genetic findings. The mechanism through which *HSD17β13* LOF variants provide a protective effect is not well elucidated. Yet, the protective effect against liver inflammation and importantly liver fibrosis is apparent not only in NASH but also in other chronic inflammatory liver diseases including alcohol-related liver disease, hepatitis C-associated fibrosis, and Wilson's disease.[10,19,20] While the mechanism is yet to be determined, the lack of reductions in liver fat observed in this study as well as in several human genetic studies may at least narrow down the plausible options, indicating that the protective mechanism is unlikely to be from metabolic shifts that reduce stored liver fat.[9,10,18,21]

It is possible that the protective effect involves changes in fatty acid metabolism, favoring pathways that limit lipotoxic lipid metabolic byproducts and thereby reducing hepatic inflammation in the setting of hepatic steatosis.

In our study, there was no clear effect on liver stiffness. Caution should be used when interpreting this result because measuring liver stiffness using FibroScan is known to be affected by ALT elevations, which were commonly present in the study subjects. Additionally, the pharmacodynamic effect through Day 71, when FibroScan was measured postdose, is likely not long enough to yield changes in liver fibrosis. Nevertheless, larger studies of longer duration will be needed to understand the impact of *HSD17β13* gene silencing on liver stiffness.

ARO-HSD was safe and well tolerated in both healthy volunteers and patients with confirmed/clinically suspected NASH, with no treatment-related SAEs, no pattern of adverse drug-related laboratory findings, and no drug discontinuations in the study. There were no clinically meaningful effects of ARO-HSD on lipids or other metabolic parameters (Supplementary Table 5). While *HSD17β13* expression is primarily in human hepatocytes, the targeted approach using ARO-HSD likely minimized any potential extrahepatic inhibition of *HSD17β13* expression, thereby mitigating any AEs that might result from such inhibition.

This study had several limitations. The study was exploratory in nature with an emphasis on biomarker pharmacodynamic endpoints. Therefore, sample sizes were small (18 patients with confirmed/clinically suspected NASH) and without placebo control in patient cohorts. However, knockdown of *HSD17β13* mRNA was consistent in the ARO-HSD 100 and 200 mg groups. Similarly, patients with confirmed/clinically suspected NASH were not randomized into any specific cohort. The lack of randomization or stratification may have contributed to imbalances between cohorts in baseline ALTs. Additionally, the pharmacologic evaluation of ARO-HSD on

HSD17β13 expression required obtaining tissue samples with liver biopsy. Samples were used to measure mRNA and protein levels to evaluate inhibition of HSD17β13 as a marker of pharmacodynamic effect as there are no known or established plasma/serum pharmacodynamic biomarkers to measure HSD17β13 activity. Given the short duration of the study and limited sample availability, evaluation of changes in histologic features of NASH was not conducted.

CONCLUSION

This clinical trial was the first to investigate therapeutic modulation of HSD17B13, a genetically validated target for the potential treatment of NASH. ARO-HSD was well tolerated at doses up to 200 mg given once (NHVs) or twice (Days 1 and 29 in patients with confirmed or clinically suspected NASH) with no significant safety or tolerability concerns noted. Reductions in liver *HSD17β13* mRNA and protein were observed and corresponded with ALT and AST reductions, which may be a clinically meaningful signal of reduced liver injury. The results of this study provide a rationale for further development of ARO-HSD in a larger phase 2 study with a longer treatment duration and histologic efficacy endpoints.

ABBREVIATIONS

AE=adverse event; ALT=alanine aminotransferase; AST=aspartate aminotransferase; BMI=body mass index; COVID-19=coronavirus disease 2019; ECG=electrocardiogram; EMA=European Medicines Agency; FDA=Food and Drug Administration; GGT=gamma-glutamyl transferase; GLP-1=glucagon-like peptide-1; ICH=International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; LOF=loss-of-function; MedDRA=Medical Dictionary for Regulatory Activities; MRI-PDFF=magnetic resonance imaging proton density fat

fraction; mRNA=messenger RNA; NAFLD=non-alcoholic fatty liver disease; NASH=non-alcoholic steatohepatitis; NHV=normal healthy volunteer; PNPLA3=patatin-like phospholipase domain containing 3; qRT-PCR=quantitative reverse transcription-polymerase chain reaction; RISC=RNA-induced silencing complex; RNAi=RNA interference; SAE=serious adverse event; SC=subcutaneous(ly); SGLT2=sodium-glucose cotransporter 2; TEAE=treatment-emergent adverse event

DATA AVAILABILITY STATEMENT

Data is available from the Study Sponsor Arrowhead Pharmaceuticals, Inc.

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Author names in bold designate shared co-first authorship.

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TABLES AND FIGURES

Tables

Table 1: Summary of Demographics and Baseline Characteristics for Patients With Confirmed or Clinically Suspected NASH

Parameter; Statistic	**Cohort 1b ARO-HSD (25 mg, Day 1, 29) (N=6)**	**Cohort 3b ARO-HSD (100 mg, Day 1, 29) (N=6)**	**Cohort 4b ARO-HSD (200 mg, Day 1, 29) (N=6)**
Demographics			
Age (years), mean (min, max)	46.2 (32, 56)	44.5 (40, 50)	42.8 (22, 61)
Male (%)	5 (83%)	5 (83%)	4 (67%)
Race - n (%)			
Asian	4 (66.7)	5 (83.3)	4 (66.7)
Native Hawaiian or Other Pacific Islander	0	1 (16.7)	0
White	2 (33.3)	0	1 (16.7)
Other	0	0	1 (16.7)
Weight (kg), mean (min, max)	96.5 (68.0, 119.7)	87.2 (65.8, 115.7)	97.5 (74.0, 117.4)
BMI (kg/cm^2), mean (min, max)	32.2 (25.7, 41.1)	29.1 (25.1, 36.5)	33.7 (28.2, 39.2)
Baseline laboratory results, ALT and AST			
Baseline ALT (U/L), mean (min, max)	45.7 (28, 69)	68.3 (30, 144)	76.0 (28, 100)
Baseline AST (U/L), mean (min, max)	21.7 (17, 27)	33.5 (22, 63)	50.3 (18, 131)
Genotype			
HSD17β13 **rs72613567, n (%)**			
T/T	5 (83)	4 (67)	3 (50)
T/TA	1 (17)	2 (33)	3 (50)

Parameter; Statistic	**Cohort 1b ARO-HSD (25 mg, Day 1, 29) (N=6)**	**Cohort 3b ARO-HSD (100 mg, Day 1, 29) (N=6)**	**Cohort 4b ARO-HSD (200 mg, Day 1, 29) (N=6)**
PNPLA3 **rs738409, n (%)**			
C/C	3 (50)	5 (83)	3 (50)
C/G	2 (33)	0	2 (33)
G/G	1 (17)	1 (17)	1 (17)

ALT=alanine aminotransferase; AST=aspartate aminotransferase; BMI=body mass index; C/C=no mutation; C/G=heterozygous; G/G=homozygous; max=maximum; min=minimum; NASH=non-alcoholic steatohepatitis; SD=standard deviation; T/T=no mutation; T/TA=heterozygous.

[a] Coded using Medical Dictionary for Regulatory Activities Version 24.0.

Table 2: Summary of TEAEs by Preferred Term Reported in ≥2 Subjects in a Cohort (NHV and Confirmed/Clinically Suspected NASH)

	Single Dose (Day 1)				
	Normal Healthy Volunteers				
	ARO-HSD				
Preferred Term	**Cohort 1 ARO-HSD (25 mg) (N=4) n (%) #**	**Cohort 2 ARO-HSD (50 mg) (N=4) n (%) #**	**Cohort 3 ARO-HSD (100 mg) (N=4) n (%) #**	**Cohort 4 ARO-HSD (200 mg) (N=4) n (%) #**	**Pooled Placebo (N=16) n (%) #**
Any TEAE	4 (100) 6	1 (25.0) 2	4 (100) 9	3 (75.0) 6	10 (62.5) 14
Headache	0	0	1 (25.0) 1	0	2 (12.5) 2
Dermatitis	0	0	0	0	2 (12.5) 2
Injection site erythema	0	0	0	2 (50.0) 2	0
	Repeat Dose (Days 1 and 29)				
	Confirmed or Clinically Suspected NASH				
	ARO-HSD				
Preferred Term	**Cohort 1b ARO-HSD (25 mg) (N=6) n (%) #**	**Cohort 3b ARO-HSD (100 mg) (N=6) n (%) #**	**Cohort 4b ARO-HSD (200 mg) (N=6) n (%) #**		
Any TEAE	3 (50.0) 8	2 (33.3) 5	4 (66.7) 10		
TEAE in 2 or more subjects [a]	--	--	--		

NASH=non-alcoholic steatohepatitis; NHV=normal healthy volunteer;

TEAE=treatment-emergent adverse event.

[a] All reported TEAEs were single event terms across various system organ classes.

Note: Coded using Medical Dictionary for Regulatory Activities Version 24.0. Subjects are counted once within each Preferred Term. Events are counted the number of times they occur.

n (%) # denotes the number of subjects, percent of subjects, and number of events in each category, respectively.

Table 3: Summary of *HSD17β13* mRNA and Protein Knockdown of ARO-HSD in Patients With Confirmed or Clinically Suspected NASH

Mean % Change from baseline (range)	**Cohort 1b ARO-HSD 25 mg (N=6)**	**Cohort 3b ARO-HSD 100 mg (N=6)**	**Cohort 4b ARO-HSD 200 mg (N=6)**	**Pooled**
Hepatic *HSD17β13* mRNA at Day 71 [a]	-56.9% (-60.5%, -50.7%)	-85.5% (-96.1%, -61.6%)	-93.4% (-98.6%, -90.8%)	-78.6% (-98.6%, -50.7%) p<0.0001
Hepatic HSD17β13 Protein at Day 71 [b,c]	< -33.8% (< -92.4%, +53.8%)	< -86.0% (-97.8%, < -63.0%)	-83.0% (-85.2, -80.8%) [d]	-63.2% (-97.8%, +53.8%) p=0.0017

LLOQ=lower limit of quantitation; mRNA=messenger RNA; NASH=non-alcoholic steatohepatitis.

[a] *HSD17β13* mRNA was measured relative to beta-actin and was calculated using the $2^{-\Delta\Delta C_T}$ method of normalization (see Supplementary Materials and Methods).

[b] HSD17B13 protein was quantified as area under the curve and normalized to vinculin protein expression (see Supplementary Materials and Methods).

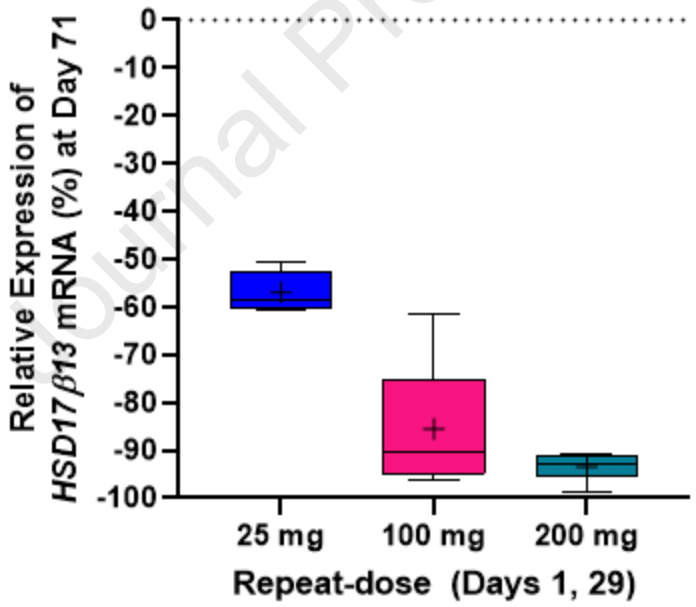
[c] Several patients had HSD17β13 protein levels at Day 71 below LLOQ, in which case LLOQ was used for calculation of means.

[d] n=2 (3 samples with baseline HSD17β13 below LLOQ, 1 sample failed assay acceptance criteria of CV ≤25%).

Due to the limited sample size from each cohort, a non-parametric Wilcoxon signed-rank test was performed to test the significance of change from baseline at Day 71 for *HSD17β13* mRNA delta Ct value and protein concentration based on pooled data.

Figures

Figure 1: Relative Expression of *HSD17β13* mRNA at Day 71 Compared to Baseline in Patients With Confirmed or Clinically Suspected NASH

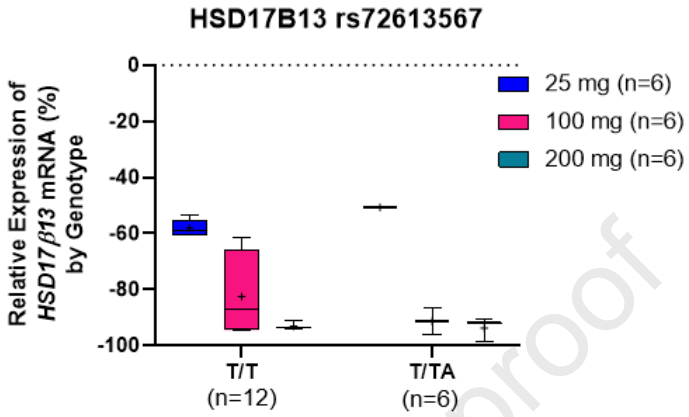


mRNA=messenger RNA; NAFLD=non-alcoholic fatty liver disease.

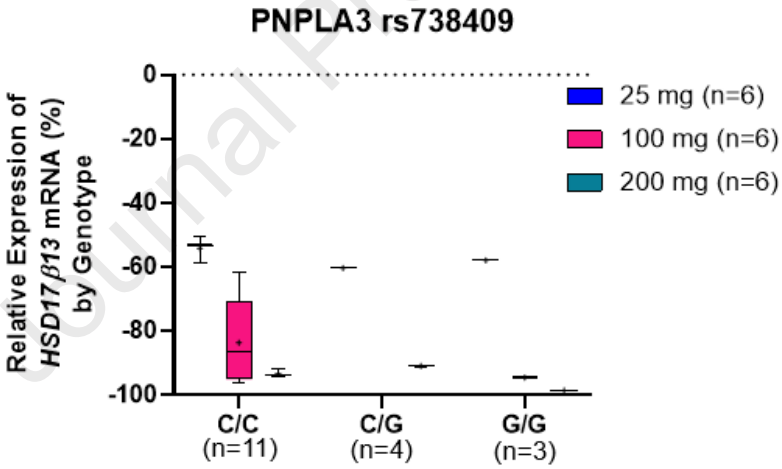
Box plot of *HSD17β13* mRNA expression at Day 71 by dose level. Box displays upper quartile, median, and lower quartile. Cross displays the mean, and whiskers extend to minimum and maximum values.

Figure 2: Messenger RNA Knockdown at Day 71 Relative to Baseline by Genotype in Patients With Confirmed or Clinically Suspected NASH

(A) *HSD17β13* rs72613567



(B) *PNPLA3* rs738409

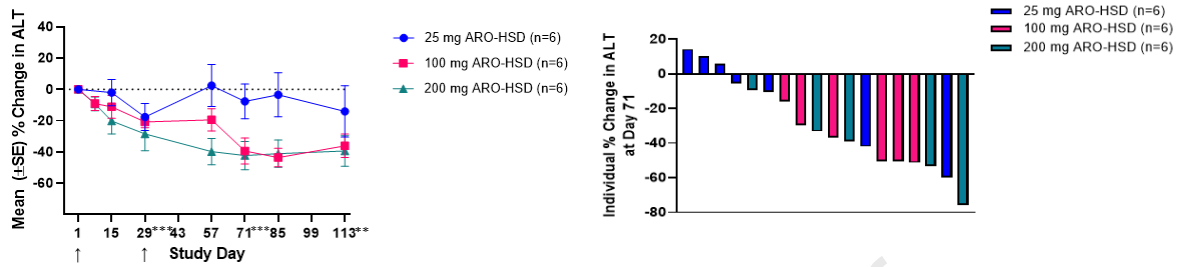


T/T=no mutation; T/TA=heterozygous; C/C=no mutation; C/G=heterozygous; G/G=homozygous.

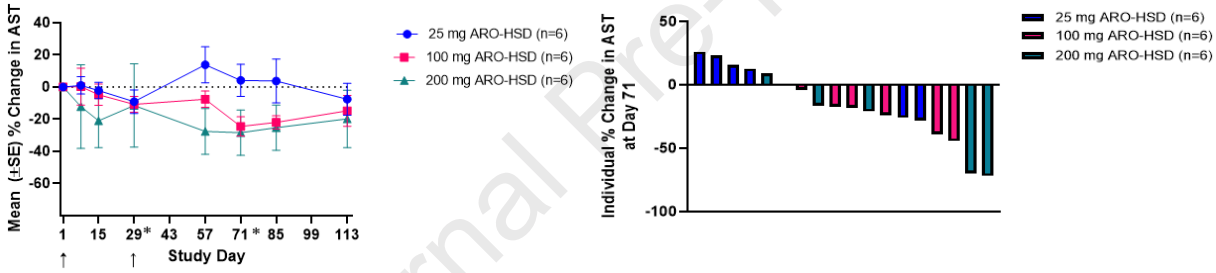
Box plot of *HSD17β13* mRNA expression at Day 71 by dose and genotype. Box displays upper quartile, median, and lower quartile. Cross mark displays mean, and whiskers extend to the minimum and maximum values.

Figure 3: Effect of ARO-HSD on Liver Enzymes in Patients With Confirmed or Clinically Suspected NASH

(A) ALT



(B) AST



ALT=alanine aminotransferase; AST=aspartate aminotransferase; SE=standard error.

Two doses of 25, 100, or 200 mg of ARO-HSD were given to patients at Day 1 and Day 29 and safety and pharmacodynamic parameters were measured through end of study, or Day 113. Duration of ALT reduction was sustained through Day 85, or 8 weeks post last dose, for 100 and 200 mg doses. Duration of AST reduction was sustained through Day 71, or 6 weeks postdose, for 100 and 200 mg doses. ***p<0.001; **p<0.01, *p<0.05 are *P* values of pooled cohorts (25, 100, and 200 mg doses) at specified timepoints (Days 29, 71, 113) using non-parametric testing (Wilcoxon signed-rank test).

ARO-HSD, an RNA Interference Therapeutic for the Treatment of Non-alcoholic Steatohepatitis: a Phase1/2 Study

Study Highlights

- HSD17B13 loss-of-function mutations are protective against alcoholic and non-alcoholic liver disease including NASH.
- ARO-HSD is a hepatocyte targeted siRNA therapeutic designed to silence HSD17B13 expression.
- ARO-HSD demonstrated a favorable safety profile in healthy volunteers and suspected NASH patients.
- Mean change from baseline at Day 71 in hepatic HSD17β13 mRNA was -93.4% (ARO-HSD 200 mg).
- Mean change in alanine aminotransferase from baseline at Day 71 was -42.3% (ARO-HSD 200 mg).