

Phase I randomized double-blind study of an RNA interference therapeutic targeting *HSD17B13* for metabolic dysfunction-associated steatohepatitis

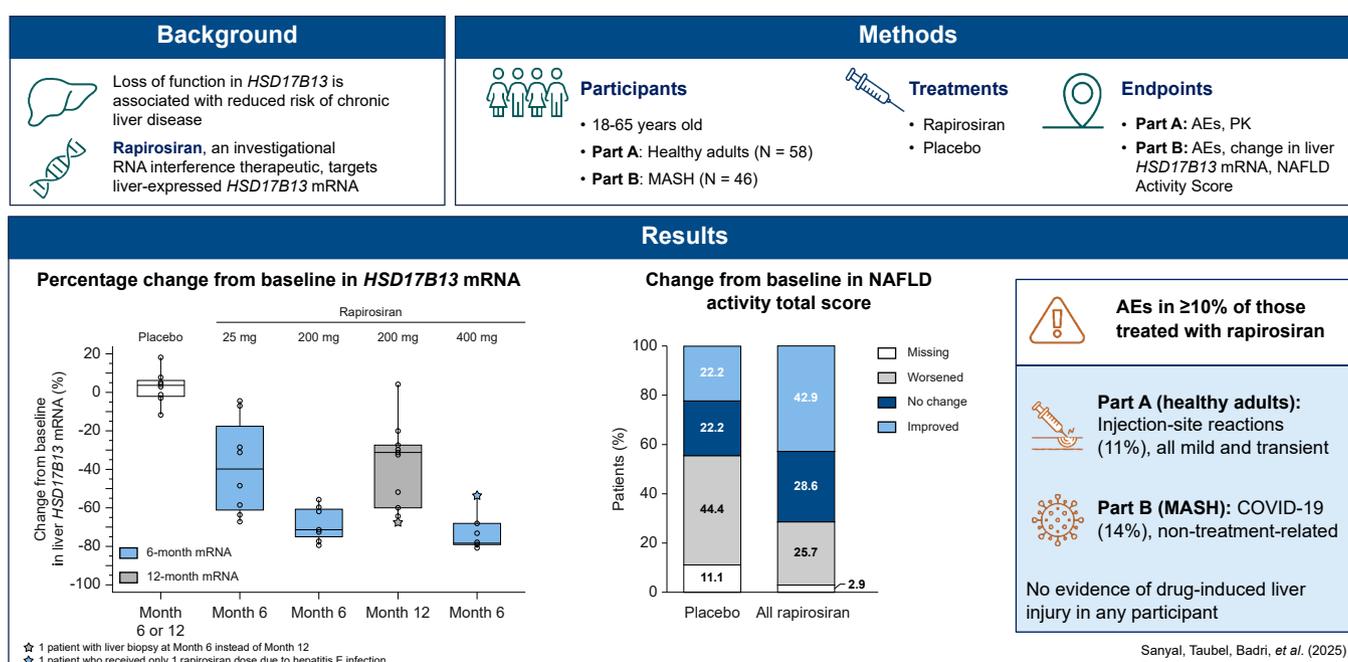
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Graphical abstract



Highlights

- Metabolic dysfunction-associated steatohepatitis is a common chronic liver disease.
- Loss-of-function *HSD17B13* gene variants are associated with reduced risk of chronic liver disease.
- Rapirosiran is an RNA interference therapeutic targeting liver-expressed *HSD17B13* mRNA.
- Rapirosiran is well tolerated and reduces liver *HSD17B13* mRNA expression.

Impact and implications

Metabolic dysfunction-associated steatohepatitis (MASH) is a prevalent chronic liver disease associated with a high burden of disease. The hydroxysteroid 17-beta dehydrogenase 13 (*HSD17B13*) gene is implicated in the pathogenesis of MASH. Rapirosiran offers a novel mechanism to treat MASH by directly reducing hepatic *HSD17B13* expression. In this phase I study, rapirosiran demonstrated an encouraging safety and tolerability profile and resulted in a robust reduction in liver *HSD17B13* mRNA expression following two subcutaneous doses. The data support further development of rapirosiran as a potential treatment option for patients with MASH, a disease for which there is only one approved pharmacological treatment.

Phase I randomized double-blind study of an RNA interference therapeutic targeting *HSD17B13* for metabolic dysfunction-associated steatohepatitis

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Journal of Hepatology 2025. vol. 83 | 838–848



Background & Aims: Genome-wide association studies have identified loss-of-function variants in the hydroxysteroid 17-beta dehydrogenase 13 gene (*HSD17B13*) associated with reduced risk of chronic liver disease. In this phase I study, we evaluated the safety, tolerability, pharmacokinetics, and pharmacodynamics of rapiroisiran, an investigational, *N*-acetylgalactosamine-conjugated small-interfering RNA targeting liver-expressed *HSD17B13* mRNA.

Methods: ALN-HSD-001 was a randomized, double-blind, placebo-controlled, multicenter study conducted in two parts. Part A evaluated single ascending subcutaneous doses of rapiroisiran or placebo in 58 healthy adults. Part B evaluated two doses, administered 12 weeks apart, in 46 adults with metabolic dysfunction-associated steatohepatitis (MASH). Patients with MASH underwent liver biopsies during screening and once post-randomization for measurement of *HSD17B13* mRNA. The primary endpoint was frequency of adverse events (AEs). Rapiroisiran plasma and urine pharmacokinetics and change from baseline in liver *HSD17B13* mRNA were secondary endpoints.

Results: In Part A, the only AE occurring in $\geq 10\%$ of rapiroisiran-treated individuals was injection-site reaction (11%); all occurrences were mild and transient. There were no treatment-related serious AEs. Plasma concentrations of rapiroisiran declined rapidly by 24 h post-dose. Across doses, rapiroisiran showed 17%–37% excretion in urine. In Part B, the only AE occurring in $\geq 10\%$ of rapiroisiran-treated patients was COVID-19 (14%; 5/36); all occurrences were deemed treatment-unrelated. There was no evidence of drug-induced liver injury in either part of the study. Rapiroisiran was associated with a dose-dependent reduction in liver *HSD17B13* mRNA in Part B, with a median reduction of 78% at 6 months in the highest-dose (400 mg) group.

Conclusions: Rapiroisiran exhibited an encouraging safety and tolerability profile, with a robust reduction in liver *HSD17B13* mRNA expression.

Clinical trial number: EUDRA-CT: 2020-000847-29; NCT: 04565717.

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Introduction

Metabolic dysfunction-associated steatohepatitis (MASH) is a chronic liver disease that can lead to progressive fibrosis, cirrhosis, and hepatocellular carcinoma.^{1–3} The prevalence of MASH in the US, Europe, and other developed countries is estimated to be between 1.5% and 6.5%.³ Weight loss is effective in reducing and even reversing MASH inflammation and fibrosis, but may be difficult to achieve and sustain.⁴ For patients with advanced disease, liver transplantation is a treatment option, but in addition to the surgical risk, it requires

life-long immunosuppression and does not preclude the development of *de novo* MASH in the transplanted liver.^{5,6}

Pharmacological treatment options for MASH are limited. Although liver transplantation is a treatment option in patients with MASH and cirrhosis, its widespread use is constrained by the limited availability of organ donors.^{7,8} Resmetirom, a thyroid hormone receptor-beta agonist, is currently the only medication approved by the US Food and Drug Administration for the treatment of adults with non-cirrhotic non-alcoholic steatohepatitis (now called MASH), with moderate to advanced fibrosis, in conjunction with diet and exercise.⁹ However, given

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<https://doi.org/10.1016/j.jhep.2025.05.031>



resmetirom's accelerated approval, which was based on improvement in liver histology, ongoing clinical outcome trials will be important to inform its clinical benefit.⁹

The etiology of MASH is multifactorial, with multiple susceptibility genes identified via genome-wide and exome-wide association studies, including genes that regulate lipid metabolism and lipid droplet biology.^{10–12} Genome-wide association studies (GWAS) have identified loss-of-function variants of the hydroxysteroid 17-beta dehydrogenase 13 gene (*HSD17B13*), which encodes a hepatic lipid droplet coat protein, that are associated with a reduced risk of chronic liver disease and with progression from steatosis to steatohepatitis. These studies suggest that for patients with MASH and underlying *HSD17B13* risk alleles, knockdown of liver-expressed *HSD17B13* mRNA may improve their liver disease.^{13–16} *HSD17B13* is expressed predominantly in the liver with minimal expression in other tissues, and its endogenous substrates are unknown.^{12–14,16} Importantly, GWAS have shown no safety signals in individuals who are homozygous for loss-of-function variants.^{14,16}

ALN-HSD (rapirosiran) is an investigational, subcutaneously administered, *N*-acetylgalactosamine (GalNAc)-conjugated RNA interference (RNAi) therapeutic agent targeting liver-expressed *HSD17B13* mRNA.¹⁷ The GalNAc ligand facilitates delivery of rapirosiran to hepatocytes through the asialoglycoprotein receptor (ASGPR) pathway. RNAi is a naturally occurring cellular mechanism for regulation of gene expression, mediated by binding of the antisense small-interfering RNA strand to the target mRNA sequence with the RNA-induced silencing complex, followed by mRNA cleavage and subsequent suppression of target protein synthesis. Through the mechanism of RNAi, rapirosiran is designed to mimic the genetic loss of *HSD17B13* function and is expected to result in protection from the hepatic injury, inflammation, and fibrosis associated with MASH. Here, we present results from the

phase I study of rapirosiran, in development for the treatment of MASH.

Patients and methods

Study design and oversight

ALN-HSD-001 was a two-part, randomized, double-blind, placebo-controlled, multicenter, single-ascending dose and multiple-dose study designed to evaluate the safety, tolerability, pharmacokinetic (PK) effects, and pharmacodynamic (PD) effects of rapirosiran in healthy adults (Part A) and adult patients with MASH (Part B). Part A was conducted at a single center in the UK between October 2020 and September 2021. Part B was conducted at 13 clinical sites across five countries (the US, the UK, Turkey, Belgium, and Bulgaria) between May 2021 and January 2023. Japanese cohorts were enrolled at the UK site. The study design is shown in Fig. 1.

The study was approved by independent ethics committees/institutional review boards at each country or site (per local regulations) and was conducted in accordance with Good Clinical Practice guidelines of the International Council for Harmonisation and the provisions of the Declaration of Helsinki. All participants provided written informed consent. A safety review committee oversaw the study. Individual participant data that support these results will not be made available. Further details regarding access to data are available at: www.vivli.org.

Participant selection

Healthy volunteers were eligible for Part A of the study if they were between the ages of 18 and 65 years, had a BMI of 18 to 28 kg/m², had normal aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin levels, and

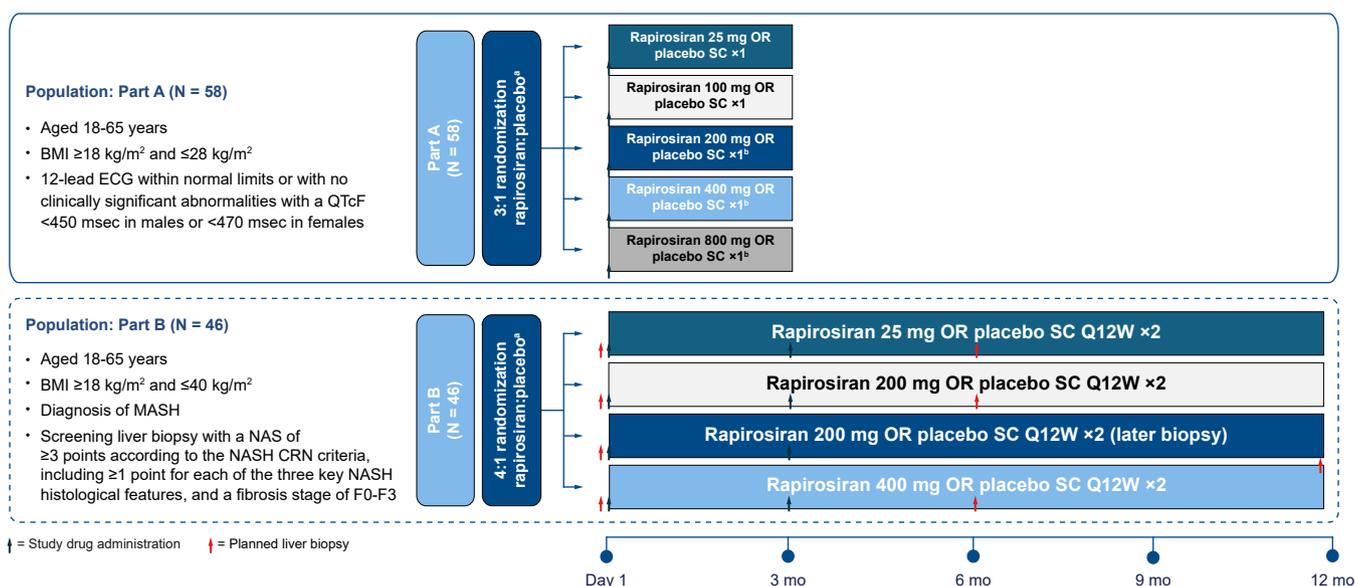


Fig. 1. Study design. ^aCohorts were enrolled sequentially. ^bIncludes a dedicated Japanese cohort. ECG, electrocardiogram; MASH, metabolic dysfunction-associated steatohepatitis; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; NASH CRN, NASH Clinical Research Network; Q12W, every 12 weeks; QTcF, Fridericia-corrected QT interval; SC, subcutaneous. (This figure appears in color on the web.)

had an estimated glomerular filtration rate of ≥ 90 ml/min/1.73 m². Patients were eligible for Part B of the study if they were between the ages of 18 and 65 years, had a BMI of 18 to 40 kg/m², and had a diagnosis of MASH documented in their medical history or a clinical suspicion for MASH based on specific criteria (*i.e.* having two or more elements of metabolic syndrome and evidence of steatosis or steatohepatitis). In addition, patients were required to have a screening liver biopsy with a non-alcoholic fatty liver disease (NAFLD) activity score (NAS) of ≥ 3 according to the NASH Clinical Research Network criteria, including ≥ 1 point for each of the three key histological features of NASH (*i.e.* steatosis, lobular inflammation, and hepatocellular ballooning), and a fibrosis stage of F0 to F3. Key exclusion criteria for Part B included an estimated glomerular filtration rate of < 45 ml/min/1.73 m², ALT level > 5 times the upper limit of normal (ULN), international normalized ratio > 1.2 , platelet count $< 140 \times 10^9/L$, alcohol-related liver disease, Model for End-stage Liver Disease score > 12 , and cirrhosis from any cause.

Procedures

In Part A of the study, healthy adults were randomized 3:1 to receive a single subcutaneous dose of rapirosiran or placebo in ascending dose groups from 25 to 800 mg. In Part B, patients with MASH were randomized 4:1 to receive rapirosiran or placebo on Day 1 and Day 85 (12 weeks apart) at one of three dose levels: 25, 200, or 400 mg (Fig. 1). The investigators, clinical study center personnel (except the site pharmacist), and participants were blinded to study drug treatment and assignment.

Serial blood samples were collected for PK analysis at 1 h pre-dosing and at 30 min and 2, 4, 6, 8, 12, 24, and 48 h after rapirosiran administration. Blood samples were evaluated for antidrug antibodies (ADA) against rapirosiran. Urine samples were collected from each participant after rapirosiran administration on Day 1, with pooled collections obtained over intervals of 0–6, 6–12, and 12–24 h. All samples were stored at -70 °C or below until analysis.

Plasma and urine PK samples were analyzed using a high-performance liquid chromatography time-of-flight mass spectrometry method with an internal standard. Only the antisense strand of rapirosiran was measured, including both the full-length antisense strand and the pharmacologically active AS(N-1)3' metabolite with the loss of one nucleotide from the 3' terminus. The activity of the AS(N-1)3' metabolite is based on *in vitro* studies. The reported duplex concentration of rapirosiran and its metabolite were based on the antisense concentration.

For patients who provided consent, genotyping was performed to detect the following single nucleotide variations: *HSD17B13* rs62305723:G vs. A, *HSD17B13* rs72613567:T vs. TA, *HSD17B13* rs80182459:GG vs. G, and *PNPLA3* rs738409:G vs. C. Risk variants are listed first; for example, *HSD17B13* rs62305723:G is the risk allele, and *HSD17B13* rs62305723:A is the protective allele.

In Part B (patients with MASH), percutaneous liver biopsies were performed at baseline and either Month 6 or Month 12, corresponding to 3 or 9 months after the second dose, respectively. Liver mRNA for *HSD17B13* quantitation was extracted from biopsies stored in RNAlater tissue storage reagent. Quantification of *HSD17B13* was performed by

reverse transcription PCR. Liver biopsies for histology were processed in formalin-fixed paraffin-embedded specimens and were read by one of eight pathologists at the central laboratory; pre- and post-biopsy specimens may not have been read by the same pathologist. Pathologists were blinded to treatment assignment.

Statistical analysis

The sample size was based on practical considerations to adequately assess safety and dose response. Statistical analyses were primarily descriptive in nature (*i.e.* mean, standard deviation, median, minimum, and maximum for continuous variables and frequencies and percentages for categorical and ordinal variables). The primary endpoint was the frequency of adverse events (AEs) for both Parts A and B. Treatment-emergent AEs (TEAE) are AEs that occur or worsen on or after the first dose of study drug through 85 days after the last dose or any AE determined to be study drug related by the investigator. Secondary endpoints in Part A included PK measures. Secondary endpoints in Part B included plasma concentrations of rapirosiran and change from baseline in liver *HSD17B13* mRNA. Change from baseline in NAS and fibrosis stage was an exploratory endpoint in Part B. The data cut-off date was March 21, 2023.

Pharmacokinetic analysis

The plasma PK parameters of rapirosiran and its metabolite were estimated by noncompartmental (NCA) methods using Phoenix WinNonlin version 7.0 (Certara LP, Princeton, NJ, USA) using data from Part A. The maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were observed from the plasma concentration-time data. Calculated PK parameters included area under the curve from time zero to the time of the last measurable concentration (AUC_{last}), area under the curve from time zero to infinity, terminal phase elimination rate constant (λ_z), half-life, renal clearance, and percentage excreted in urine. Day 1 C_{max} and AUC of rapirosiran after a single dose for each cohort were evaluated using a power model for dose proportionality. The power model is described as $y = \alpha \times \text{Dose}^\beta$, where y , α , and β correspond to the PK variable, proportionality constant, and exponent, respectively. The exponent β in the power model was estimated by regressing the natural log-transformed PK variable on the natural log-transformed dose. Dose proportionality is implied when the estimated 90% confidence interval for β contains 1.

Results

Part A enrolled 58 healthy adults (including 18 Japanese participants) and Part B enrolled 46 patients with MASH. The disposition of the participants is shown in Fig. 2. Baseline characteristics were largely comparable between the total rapirosiran and placebo groups in both parts (Table 1). In Part B, the mean (range) age was 55.6 (34–64) years for patients receiving rapirosiran and 52.3 (35–64) years for patients receiving placebo. A total of 56% of patients receiving rapirosiran and 44% of patients receiving placebo were male. Mean baseline BMIs were similar between the rapirosiran and placebo groups (33.5 and 34.9 kg/m², respectively). Mean

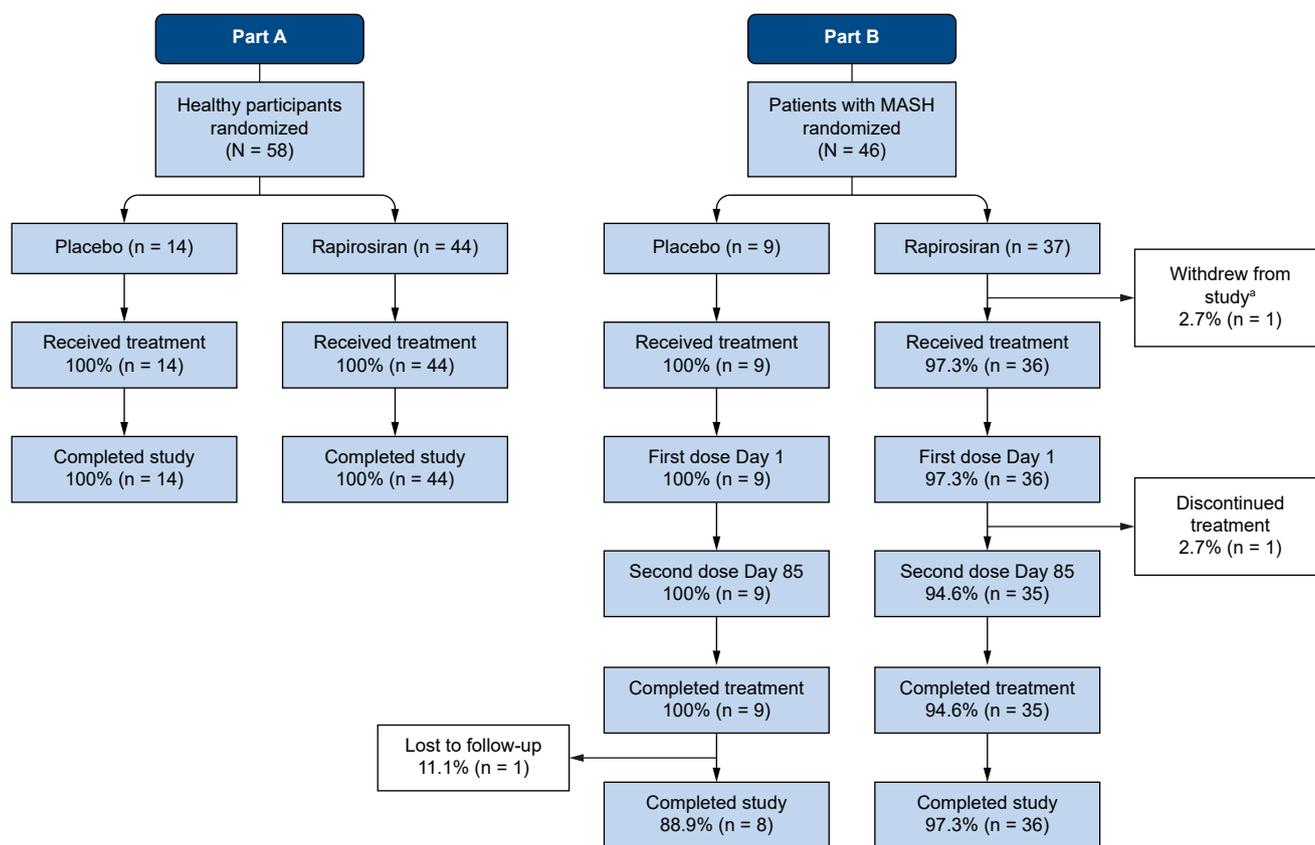


Fig. 2. Disposition of healthy participants (Part A) and patients with metabolic dysfunction-associated steatohepatitis (MASH; Part B). ^aOne patient with hepatitis E withdrew from the study due to mildly increased liver function tests. MASH, metabolic dysfunction-associated steatohepatitis.

baseline NAS (4.4 and 4.2, respectively) and mean fibrosis stage (1.7 and 1.6, respectively) were similar between the rapirosiran and placebo groups. In Part B, no patients had homozygous protective alleles for any of the three variants of

HSD17B13 (rs62305723:G, rs72613567:T, and rs80182459:GG); similar proportions of patients in the placebo and rapirosiran groups were heterozygous or homozygous for the *PNPLA3* rs738409:G (I148M) risk allele (Table S1).

Table 1. Demographics and baseline characteristics.

Part A: Healthy participants	Placebo (n = 14)	Rapirosiran (n = 44)
Age, mean (range), years	27.2 (19–43)	29.4 (20–41)
Male sex, n (%)	8 (57)	25 (57)
White race, n (%)	8 (57)	25 (57)
Japanese, n (%)	4 (29)	14 (32)
BMI, mean (SD), kg/m ²	22.1 (1.9)	22.0 (1.7)
ALT, mean (SD), IU/L ^a	18.1 (6.6)	18.5 (8.7)
AST, mean (SD), IU/L ^b	16.1 (2.4)	17.9 (4.8)
Part B: Patients with MASH	Placebo (n = 9)	Rapirosiran (n = 36) ^c
Age, mean (range), years	52.3 (35–64)	55.6 (34–64)
Male sex, n (%)	4 (44)	20 (56)
White race, n (%)	7 (78)	28 (78)
BMI, mean (SD), kg/m ²	34.9 (4.6)	33.5 (3.7)
ALT, mean (SD), IU/L ^d	57.6 (33.9)	49.6 (23.5)
AST, mean (SD), IU/L ^e	41.0 (21.9)	36.9 (17.9)
Cholesterol, mean (SD), mmol/L	5.47 (1.2)	4.84 (1.1)
Triglycerides, mean (SD), mmol/L	2.85 (1.4)	1.84 (1.1)
Hemoglobin A1c, mean (SD), %	6.5 (0.9)	6.4 (1.2)
NAFLD activity score, mean (SD)	4.2 (0.8)	4.4 (1.0)
Fibrosis stage, mean (SD)	1.6 (0.7)	1.7 (0.8)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; MASH, metabolic dysfunction-associated steatohepatitis; NAFLD, non-alcoholic fatty liver disease.

^aALT normal range: 10–35 U/L (females), 10–50 U/L (males).

^bAST normal range: 0–31 U/L (females), 0–37 U/L (males).

^cOne patient with hepatitis E withdrew from the study prior to receiving the first dose due to increased values on liver function tests.

^dALT normal range: ≤33 U/L (females), ≤41 U/L (males).

^eAST normal range: ≤31 U/L (females), ≤37 U/L (males).

AEs are summarized in Table 2. In Part A, all AEs in healthy participants treated with rapirosiran were mild to moderate in severity. The most common AE was injection-site reaction, with symptoms of bruising or blanched skin at the injection site in 11% of patients (n = 5/44) in the rapirosiran group compared with no cases in the placebo group. There was only one serious AE, an event of tonsillitis in one participant treated with rapirosiran; this event was deemed not related to the study drug. There were no AEs of clinical interest, including elevations in ALT or AST (defined as >3x the ULN if normal at baseline or >3x baseline if elevated at baseline), in Part A. In Part B, the majority of AEs were mild or moderate in severity. The only AEs occurring in ≥10% of patients in either group were COVID-19 in 14% (n = 5/36) of patients in the total rapirosiran group, all deemed not related to the study drug, and upper respiratory tract infection in 22% of patients (n = 2/9) in the placebo group, all deemed not related to the study drug. There was one treatment-emergent serious AE of appendicitis in one participant treated with rapirosiran, which was deemed not related to the study drug. A single AE of clinical interest was reported in Part B: one patient treated with rapirosiran discontinued the study drug due to an ALT level >3x the ULN and an AST level >5x the ULN on Day 68, both of which normalized by Day 85. This patient tested positive for hepatitis E, and the investigator graded the event as mild and considered it not related to rapirosiran but related to hepatitis E.

There were no treatment-related AEs in Part A. In Part B, there were five treatment-related AEs reported in 4 (11%)

patients treated with rapirosiran: in the 200 mg dose group, Basedow's disease (also known as Graves' disease; diagnosed on study Day 217 and considered moderate in severity), chills and abnormal gastrointestinal sounds (on Day 1 with no recurrence with the second dose), and mild injection-site reaction, and in the 25 mg dose group, vasculitis rash of mild severity localized to the top of the right foot diagnosed as a discrete hyperpigmented macule consistent with post-inflammatory hyperpigmentation on Day 38 that was resolving. Two AEs were deemed treatment-related in 2 (22%) patients treated with placebo: abdominal pain and nausea. In Part B, 2 (6%) patients treated with rapirosiran reported injection-site reaction AEs. There was no evidence of drug-induced liver injury in either part of the study. Two cases of treatment-emergent ADAs were noted in Part A (n = 1, 100 mg dose) and Part B (n = 1, 200 mg dose). Overall, no safety concerns were identified based on the safety data observed in the study.

Pharmacokinetics results from Part A are summarized in Table S2. Rapirosiran was absorbed with a median T_{max} of 4.0 to 6.1 h. Plasma concentrations of rapirosiran declined rapidly and were undetectable by 48 h in most participants (Fig. S1), with a mean terminal half-life ranging between 4.2 and 5.7 h. The active metabolite, AS(N-1)3' ALN-HSD, was detectable at doses ≥100 mg and showed a median T_{max} of 4.0 to 8.0 h, and the levels in plasma were undetectable by 24 h in most participants (Fig. S2). The half-life of AS(N-1)3' ALN-HSD (mean value 5.5 to 6.8 h) was similar to that of rapirosiran. The

Table 2. Treatment-emergent adverse events.

Part A: Healthy participants ^a	Placebo (n = 14)	Rapirosiran (n = 44)
TEAE ^b	3 (21)	17 (39)
AEs reported in ≥10% of either group and at a higher rate in rapirosiran group than placebo group		
Injection-site reaction ^c	0 (0)	5 (11)
Serious AE	0 (0)	1 (2)
Severe AE	0 (0)	0 (0)
Treatment-related AE	0 (0)	0 (0)
TEAE of clinical interest ^d	0 (0)	0 (0)
TEAE leading to study withdrawal	0 (0)	0 (0)
Death ^e	0 (0)	0 (0)
Part B: Patients with MASH	Placebo (n = 9)	Rapirosiran (n = 36) ^f
TEAE	7 (78)	29 (81)
AEs reported in ≥10% of either group and at a higher rate in rapirosiran group than placebo group		
COVID-19	0 (0)	5 (14)
Serious TEAE	0 (0)	1 (3) ^g
Severe TEAE	0 (0)	2 (6) ^h
Treatment-related AE	2 (22)	4 (11)
TEAE of clinical interest ^d	0 (0)	1 (3) ⁱ
TEAE leading to study drug discontinuation	0 (0)	1 (3) ^j
TEAE leading to study withdrawal	0 (0)	0 (0)
Death ^e	0 (0)	0 (0)

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; MASH, metabolic dysfunction-associated steatohepatitis; TEAE, treatment-emergent adverse event; ULN, upper limit of normal.

All data reported are n (%).

^aSafety data reported over the 3-month double-blind period.

^bTEAE is defined as an AE that occurs or worsens on or after the first dose of study drug through 85 days after the last dose or any AE determined to be study drug related by investigator.

^cIncludes bruising, blanching, and blanched skin at injection site.

^dAEs of clinical interest: ALT or AST >3 × ULN for participants with normal ALT and AST values at baseline, and >3 × baseline in patients with MASH with elevated ALT or AST at baseline, or severe or serious ISRs, ISRs associated with a recall phenomenon (reaction at the site of a prior injection with subsequent injections), or those that led to temporary dose interruption or permanent discontinuation of rapirosiran.

^eAll deaths are included regardless of whether they were treatment-emergent.

^fOne patient with hepatitis E withdrew from the study prior to receiving the first dose due to increased values on liver function tests.

^gOne patient had appendicitis.

^hOne patient had appendicitis, and one patient had a tooth infection.

ⁱOne patient had increased liver enzyme levels (due to hepatitis E).

^jNone of the ISR cases met the criteria for clinical interest.

metabolite-to-parent ratios for the C_{max} ($MR_{C_{max}}$) and area under the concentration-time curve AUC_{last} ($MR_{AUC_{last}}$) were low; both $MR_{C_{max}}$ and $MR_{AUC_{last}}$ had mean values ranging from 0.03 to 0.05. For rapirosiran, the C_{max} and the AUC_{last} in plasma increased in a slightly greater than dose-proportional manner over the 25 to 800 mg dose range. The power model estimates for the slope (β) (90% confidence intervals) were 1.22 (1.12–1.33) for C_{max} and 1.29 (1.19–1.38) for AUC_{last} . Across doses, the mean percentages of rapirosiran and AS(N-1)3' ALN-HSD in the urine after 24 h urine collection were 17% to 37% and <2%, respectively, exhibiting a slight increase with dose. Plasma and urine PK parameters were similar between Japanese and non-Japanese participants. In Part B, where sparse plasma samples were collected, rapirosiran concentrations on Day 85 were similar to those obtained on Day 1.

Rapirosiran was associated with dose-dependent reduction of *HSD17B13* mRNA at Month 6, 3 months after the second dose of rapirosiran (Fig. 3). The median percentage changes in *HSD17B13* mRNA at Month 6 relative to baseline were -39.8%, -71.4%, and -78.3% in patients receiving 25 mg, 200 mg, and 400 mg of rapirosiran, respectively, compared with 4.7% in patients receiving placebo (Fig. 3B). One patient in the 400 mg dose group received only the first dose of

rapirosiran due to a hepatitis E infection and had a change in *HSD17B13* mRNA of -53.7% at Month 6. At Month 12, 9 months after the second dose of rapirosiran, the median percentage change in *HSD17B13* mRNA was -30.6% in patients receiving 200 mg of rapirosiran compared with -1.1% in patients receiving placebo (Fig. 3B).

In Part B, rapirosiran was associated with numerically lower ALT and AST levels over time compared with placebo (Figs 4 and 5). At Month 6 (Day 169), the timepoint at which suppression is expected to be at its maximum, the mean (SEM) ALT in patients receiving rapirosiran was 37.7 (3.9) U/L compared with 83.8 (21.2) U/L in patients who received placebo (Fig. 4A); the corresponding mean (SEM) percentage changes in ALT from baseline were -19.0% (6.2%) for rapirosiran vs. 37.3% (15.5%) for placebo (Fig. 4B). The mean (SEM) levels of AST at Day 169 were 31.7 (3.0) U/L in patients treated with rapirosiran compared with 58.6 (12.7) U/L in those receiving placebo (Fig. 5A), representing mean (SEM) percentage changes from baseline of -11.5% (5.2%) and 43.6% (15.1%), respectively (Fig. 5B).

When liver histology was evaluated, rapirosiran was associated with numerically lower NAS and fibrosis stages over 6 or 12 months relative to placebo (Fig. 6). The NAS

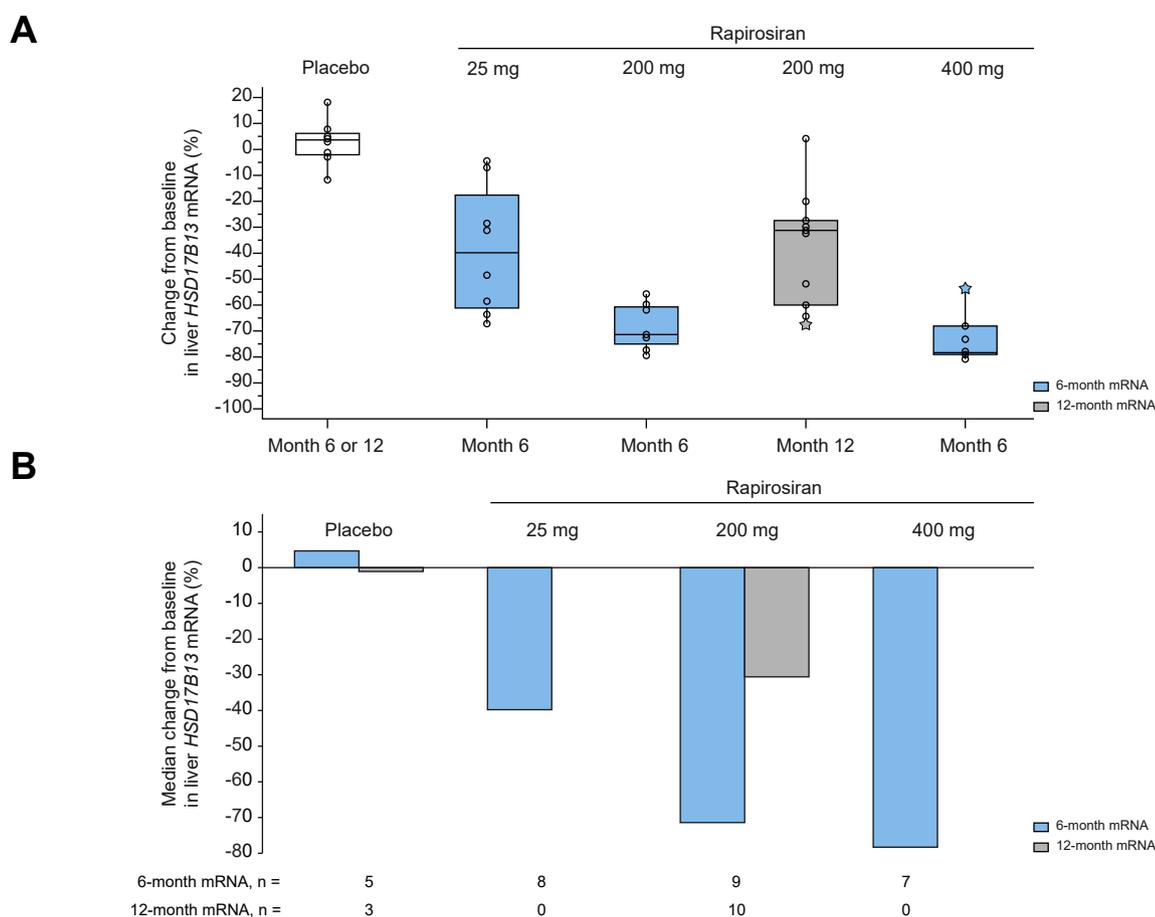


Fig. 3. *HSD17B13* mRNA percentage change in liver biopsies at Month 6 and Month 12 in patients with metabolic dysfunction-associated steatohepatitis. (A) Box plot, (B) Column plot. No patients had homozygous protective alleles for any of the three variants of *HSD17B13*. 12-month mRNA was not evaluated in the rapirosiran 25 mg or 400 mg dose groups. Boxplot: The gray star (rapirosiran, 200 mg, Month 12) indicates one patient who had liver biopsy at Month 6 instead of Month 12, and the green star (rapirosiran, 400 mg, Month 6) indicates one patient who received only a first dose of rapirosiran due to hepatitis E infection and had a change in *HSD17B13* mRNA of -53.7% at Month 6.

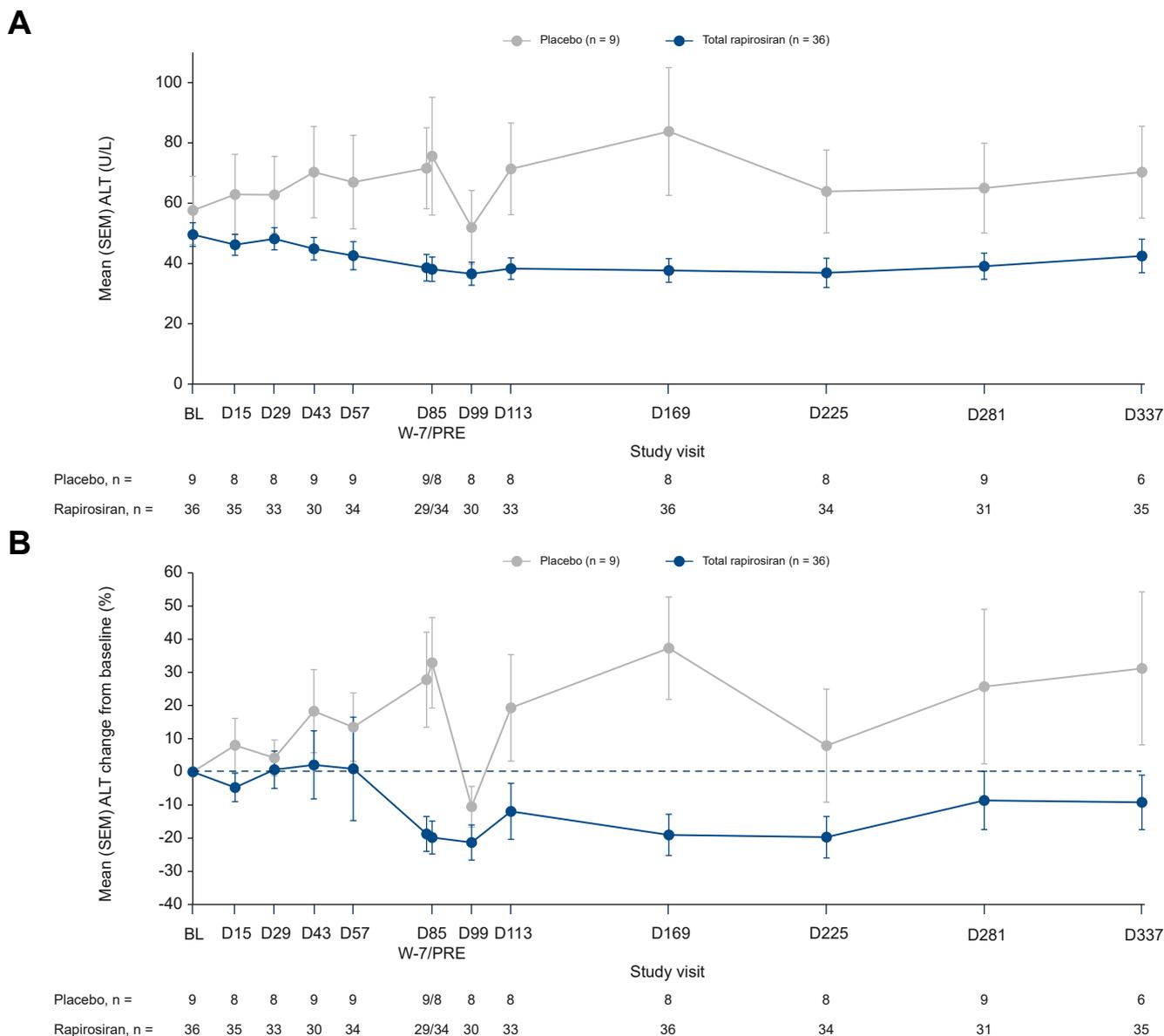


Fig. 4. ALT levels over time in patients with metabolic dysfunction-associated steatohepatitis. (A) Mean (SEM) actual values. (B) Mean (SEM) percentage change from baseline. ALT, alanine aminotransferase; BL, baseline; D, Day; W-7/PRE, within 7 days/pre-dose.

result was largely due to reductions in lobular inflammation and ballooning.

Non-invasive blood-based diagnostic tests showed a reduction in circulating biomarkers such as cytokeratin 18 fragment M65 (mean [SD] percentage change from baseline: -21.8% [31.0%] vs. 77.0% [200.0%]) and fragment M30 (-14.9% [50.2%] vs. -0.1% [69.6%]) at Month 6 for rasirosiran relative to placebo. At Month 6, enhanced liver fibrosis score increased (mean [SD] change from baseline: 0.21 [0.78] vs. -0.01 [0.49]) and FibroTest scores were relatively unchanged (mean: -0.018 [0.069] vs. -0.038 [0.079]) for rasirosiran compared with placebo.

Anthropometric and metabolic measurements over time (BMI, weight, hemoglobin A1c, lipid parameters) are presented in [Figs. S3–9](#).

Discussion

The purpose of this study was to characterize the PK, PD, and safety and tolerability profile of rasirosiran to support dose selection of rasirosiran for phase II clinical trials. Rasirosiran had an acceptable safety profile in healthy adults and in patients with MASH. The majority of AEs were mild or moderate, and no treatment-related serious or severe AEs were reported during the study.

In this phase I study, rasirosiran was rapidly absorbed in healthy adults over a wide range of doses and elicited a robust mRNA reduction in patients with MASH. Following subcutaneous administration of rasirosiran, C_{max} was reached by 4 to 6 h post-dose. The rapid decline in plasma concentrations of rasirosiran and its metabolite to lower limits of quantification

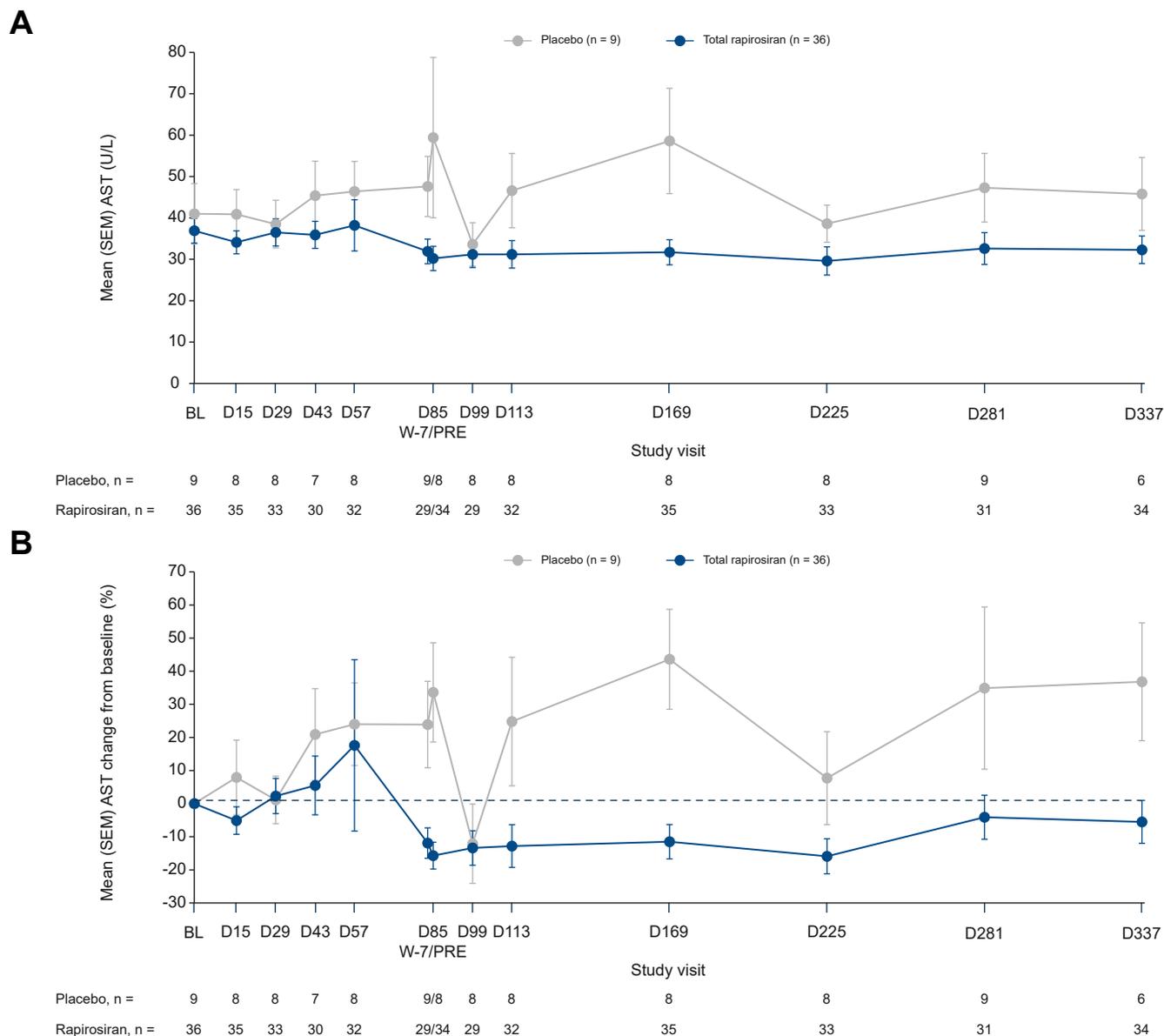


Fig. 5. AST levels over time in patients with metabolic dysfunction-associated steatohepatitis. (A) Mean (SEM) actual values. (B) Mean (SEM) percentage change from baseline. AST, aspartate aminotransferase; BL, baseline; D, Day; W-7/PRE, within 7 days/pre-dose.

by 48 h and their short plasma half-life can be attributed to efficient ASGPR-mediated uptake into hepatocytes, the site of RNAi activity.¹⁸ As the rapirosiran dose increases, rapirosiran plasma concentrations seem to transiently saturate ASGPR, resulting in slightly more than dose-proportional plasma-exposure increases. The transient nature of saturation is attributable to the rapid recycling of ASGPR back to the surface of the hepatocyte to engage with new GalNAc conjugates, resulting in plasma concentrations declining below the lower limit of quantification within 48 h after dosing. This PK profile is consistent with other siRNA molecules, such as vutrisiran and cemdisiran.^{19,20} As has been observed with vutrisiran and cemdisiran, the PK profiles of rapirosiran were similar between Japanese and non-Japanese participants.^{19,20} To date, there have been no reports of differences in expression and activity of ASGPR or endo- and exonucleases between Japanese and

non-Japanese participants. In addition, rapirosiran and its metabolite plasma PK were similar between healthy participants and patients with MASH.

The mean percentage of rapirosiran excreted in the urine was low (17% to 37%), while <2% was recovered in urine as AS(N-1)3' ALN-HSD in healthy participants. The percentage of urine excretion for rapirosiran increased with dose, suggesting that transiently higher plasma concentrations due to ASGPR saturation results in a slightly greater amount of rapirosiran available for filtration by the kidneys. Even at the highest single dose of 800 mg, urine recovery of rapirosiran was low (33%), indicating that the rate of hepatic uptake of siRNA through ASGPR far exceeds the glomerular filtration rate. Similarly low renal excretion has been observed for different GalNAc-siRNA conjugates designed for different targets.²¹ Therefore, since the renal route is not a major elimination pathway, renal

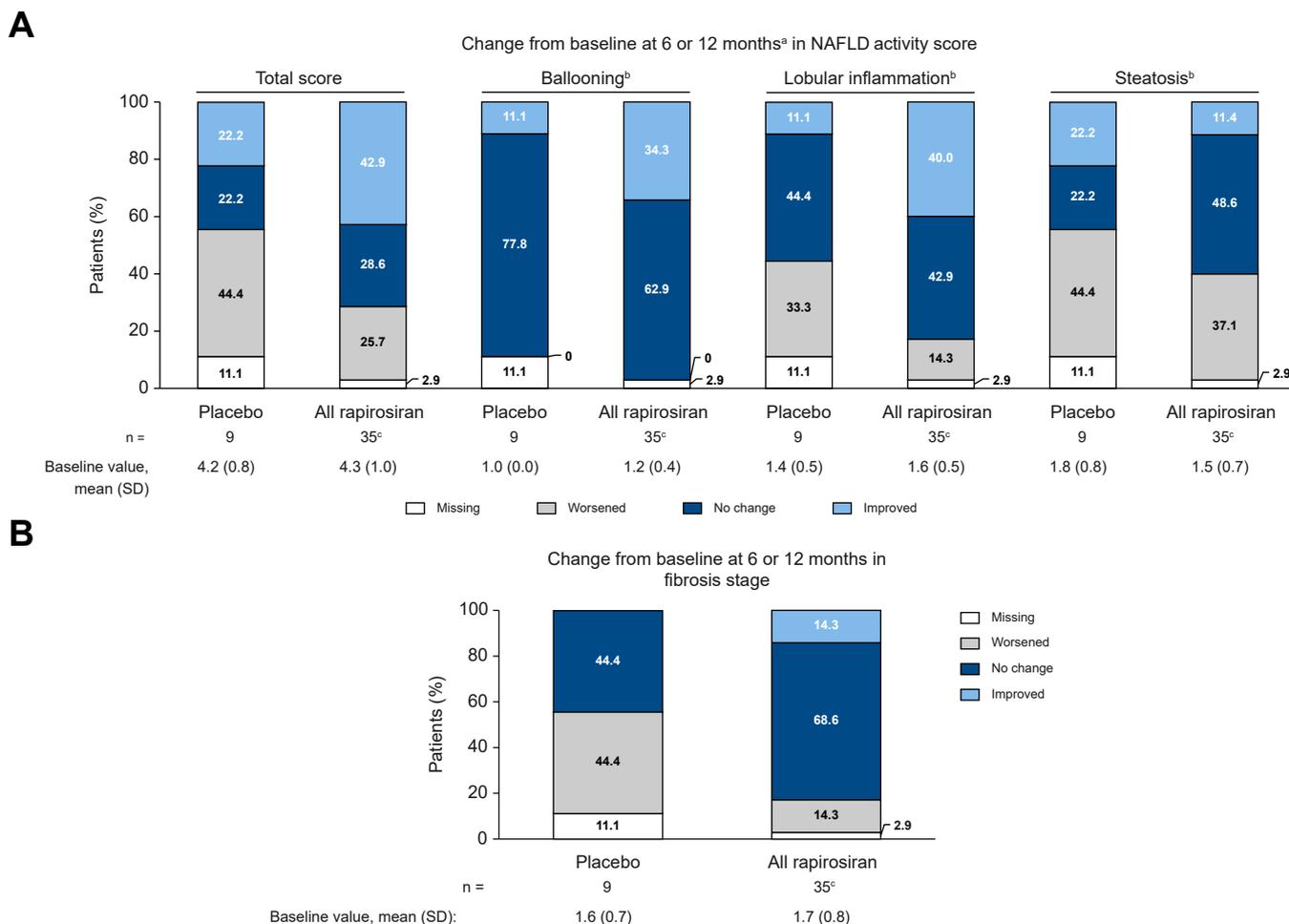


Fig. 6. Liver histology changes in patients with metabolic dysfunction-associated steatohepatitis. (A) Biopsy-derived NAFLD activity score and (B) biopsy-derived fibrosis stage: mean changes from baseline at 6 or 12 months. ^aSix-month biopsies were available in the 25 mg, 200 mg, and 400 mg cohorts. Twelve-month biopsies were available in only one 200 mg cohort, except for one patient who underwent liver biopsy at Month 6 instead of Month 12. ^bSubcomponent of NAFLD total activity score. ^cSecond biopsy visit was cancelled in one patient out of 36 in the rapirosoiran group. Worsened: change from baseline >0. Improved: change from baseline <0. NAFLD, non-alcoholic fatty liver disease.

impairment is not anticipated to have a major impact on the PK of rapirosoiran and AS(N-1)3' ALN-HSD. The incidence of treatment-emergent ADAs was low in those treated with rapirosoiran, occurring in 2.3% of healthy participants and 2.8% of patients with MASH, at doses of 100 and 200 mg, respectively.

Across dose groups, most participants receiving rapirosoiran had a marked reduction from baseline in liver *HSD17B13* mRNA compared with those receiving placebo. The prolonged PD effect on HSD mRNA lasting for ≥ 6 months is particularly noteworthy given the short plasma half-life of rapirosoiran and its metabolite. This indicates that rapirosoiran has a long half-life in the liver, enabling it to exert its inhibitory effect. Similarly transient plasma PK and prolonged PD were also observed for all of the other five approved GalNAc siRNAs, including lumasiran, inclisiran, nedosiran, vutrisiran, and givosiran.^{19,22–25}

Rapirosoiran was associated with numerically lower ALT and AST levels and liver biopsy-derived NAS and liver fibrosis stage over 6 or 12 months relative to placebo. The NAS results were largely driven by reductions in lobular inflammation and ballooning. The lack of an apparent effect on steatosis with

rapirosoiran is consistent with what has been observed in GWAS, where loss-of-function *HSD17B13* variants have not been associated with a reduced risk of steatosis,^{13,15,26} such that the pattern of NAS histologic changes matches what would be expected for an RNAi therapeutic targeting *HSD17B13*. That said, the ability to draw any meaningful conclusions with regard to changes in liver histology is limited by the small sample size, the early biopsy timepoints (e.g. most taken only 3 months after the last dose), the heterogeneous MASH population inclusive of fibrosis stage F0–F3 with a NAS ≥ 3 , and the inclusion of participants agnostic of genotype (i.e. analyses not restricted to *HSD17B13* risk allele homozygotes). The impact of *HSD17B13* mRNA liver knockdown on liver histology will be more definitively answered by the ongoing phase II study (NCT05519475), evaluating rapirosoiran in patients with fibrosis stage F2 or F3 MASH who are homozygous for *HSD17B13* risk alleles.

In the current study, no patient was homozygous for loss-of-function *HSD17B13* variants (i.e. homozygous for protective allele carriers who might be unlikely to derive benefit from targeting *HSD17B13*). However, multiple participants

were heterozygous for at least one of the *HSD17B13* variants, for whom the impact of *HSD17B13* knockdown may be tempered compared with individuals who are homozygous for all three *HSD17B13* risk alleles. The global prevalence (which takes into account heterozygotes and homozygotes) of the most studied *HSD17B13* variant (rs72613567) is 18%.²⁷ Depending on the population studied, the prevalence of loss-of-function *HSD17B13* variants ranges from 5% in Africa to 34% in East Asia.²⁸ Based on an analysis of GWAS, patients with the *PNPLA3* I148M risk allele might show the largest benefit from targeting *HSD17B13*.¹³ Conversely, some studies have found no interaction effect between the *HSD17B13* rs72613567 and *PNPLA3* rs738409 alleles.^{15,26}

Given the increasing burden of MASH, new treatments are urgently needed.^{1–3} Potential advantages of an RNAi approach to the treatment of MASH include the ability to achieve

targeted liver gene suppression with infrequent dosing and a subcutaneous route of drug administration.

Limitations of our study inherent to most phase I studies include the small number of participants and relatively short duration of follow-up. As previously noted, in Part B, some patients with NAS scores of 3 or 4 and/or F0 fibrosis were included; these patients would generally be excluded from later-phase studies. In addition, assessment of histologic findings in liver biopsies was not performed using a panel of pathologists, and pre- and post-biopsy assessments may not have been performed by the same pathologist, which would have increased confidence in the results.

In conclusion, rapiroiran exhibited an encouraging safety and tolerability profile in the phase I ALN-HSD-001 study, with robust reduction in liver *HSD17B13* mRNA expression, supporting further clinical development.

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Abbreviations

ADA, antidrug antibody; AEs, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ASGPR, asialoglycoprotein receptor; AUC_{0–last}, area under the curve from time zero to the time of the last measurable concentration; C_{max}, maximum plasma concentration; GalNAc, *N*-acetylgalactosamine; GWAS, genome-wide association study; *HSD17B13*, hydroxysteroid 17-beta dehydrogenase 13; MASH, metabolic dysfunction-associated steatohepatitis; MR, metabolite-to-parent ratios; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; PD, pharmacodynamic(s); PK, pharmacokinetic(s); RNAi, RNA interference; TEAE, treatment-emergent adverse event; T_{max}, time to reach C_{max}; ULN, upper limit of normal.

Financial support

Funding for the completion of the study and writing of the manuscript was provided by Alnylam Pharmaceuticals and Regeneron Pharmaceuticals, Inc. The sponsors were involved in study design, collection, analysis and interpretation of data, development of the manuscript, and in the decision to submit the article for publication.

Conflict of interest

AJS: Holds stock options in Genfit S.A., Tiziana, Indalo, Durect, Rivus, NorthSea, Inversago, and Galmed Pharmaceuticals; Consultant to AstraZeneca, Salix, Takeda, Gilead Sciences, Inc., Terns Pharmaceuticals, Merck, Madrigal Pharmaceuticals, NGM Biopharmaceuticals, Inc., Sagimet Biosciences, Boehringer Ingelheim, Boston Pharmaceuticals, Eli Lilly, Inventiva, Akeru Therapeutics, 89bio, Inc., Novo Nordisk, Pfizer, Amgen, Genentech, Regeneron, Alnylam, Hanmi, LG Chem, Histoindex, Thera Technologies, Intercept Pharmaceuticals, Target-RWE, Surrozen, Zydus, Path AI, Exalenz, and Genfit S.A. Serves on scientific advisory board for Histoindex, Pelayo and Avant Sante (funds paid to university). Royalties received from Elsevier and UpToDate. Grant support to institution from Gilead Sciences, Inc., Salix, Bristol Myers Squibb, Pfizer, Novo Nordisk, Viking, Akeru, Eli Lilly, Boehringer Ingelheim, Intercept Pharmaceuticals, Merck, Takeda, AstraZeneca, Hanmi, Mallinckrodt, and Novartis Pharmaceuticals. JT: Employee and shareholder of Richmond Pharmacology, which received a commercial remuneration for conducting elements of the trial. PB: Employee of Alnylam Pharmaceuticals and holds shares and stock options in Alnylam Pharmaceuticals. SB: Employee of Alnylam Pharmaceuticals and holds shares and stock options in Alnylam Pharmaceuticals. NM: Employee of Alnylam Pharmaceuticals and holds shares and stock options in Alnylam Pharmaceuticals. WZ: Employee of Alnylam Pharmaceuticals and holds shares and stock options in Alnylam Pharmaceuticals. SD: Employee of Regeneron Pharmaceuticals, Inc., and holds stock options in Regeneron Pharmaceuticals, Inc. FK: Employee of Alnylam Pharmaceuticals and holds shares and stock options in Alnylam Pharmaceuticals. BAO: Employee of Regeneron Pharmaceuticals, Inc. and holds stock options in Regeneron Pharmaceuticals, Inc. JMG: Employee of Alnylam

Pharmaceuticals and holds shares and stock options in Alnylam Pharmaceuticals.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Study concept and design: BAO, PB, JT, AJS. Acquisition of data: SB, JT. Analysis and interpretation of data: JMG, FK, PB, NM. Drafting of the manuscript: JMG, PB. Critical revision of the manuscript for important intellectual content: JMG, FK, PB, JT, AJS. Statistical analysis: WZ. Study supervision: JMG, JT.

Data availability

Individual participant data that support these results will not be made available. Further details regarding access to data are available at: www.vivli.org.

Acknowledgements

We thank all the patients and their families for participating in the study. Medical writing and editorial assistance were provided by Peloton Advantage, LLC, an OPEN Health company, in accordance with Good Publication Practice (GPP3) guidelines and were funded by Alnylam Pharmaceuticals and Regeneron Pharmaceuticals, Inc.

Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2025.05.031>.

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Author names in bold designate shared co-first authorship

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Keywords: RNAi therapeutics; non-alcoholic fatty liver disease; metabolic dysfunction-associated steatohepatitis; liver diseases; *HSD17B13*.

Received 21 October 2024; received in revised form 8 May 2025; accepted 15 May 2025; available online 27 June 2025

Supplemental information

Phase I randomized double-blind study of an RNA interference therapeutic targeting *HSD17B13* for metabolic dysfunction-associated steatohepatitis

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therapeutic targeting *HSD17B13* for metabolic dysfunction-
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Fig. S1. Fig. S1. Mean plasma rapirosiran concentrations over time.

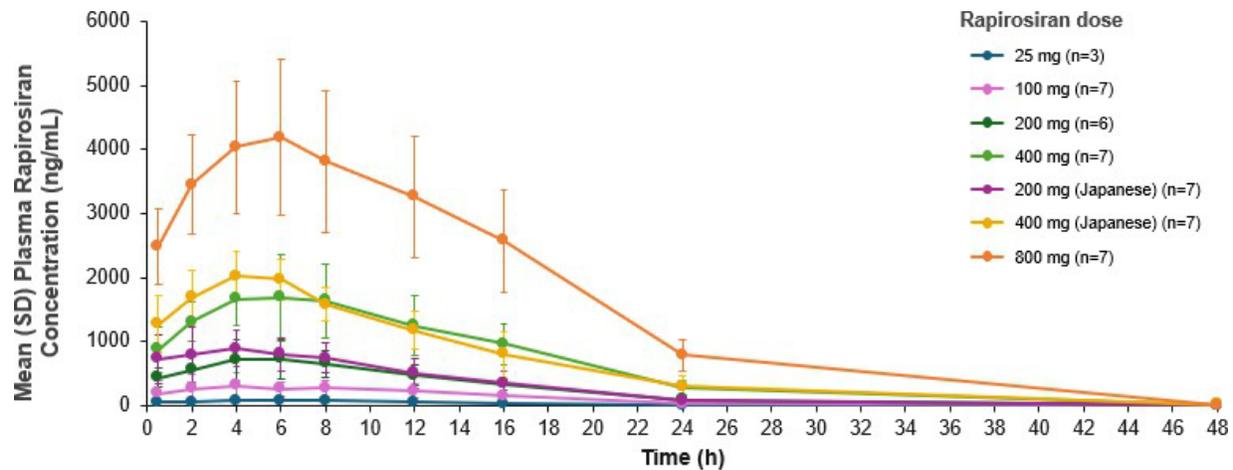


Fig. S2. Mean plasma AS(N-1)3' ALN-HSD concentrations over time.

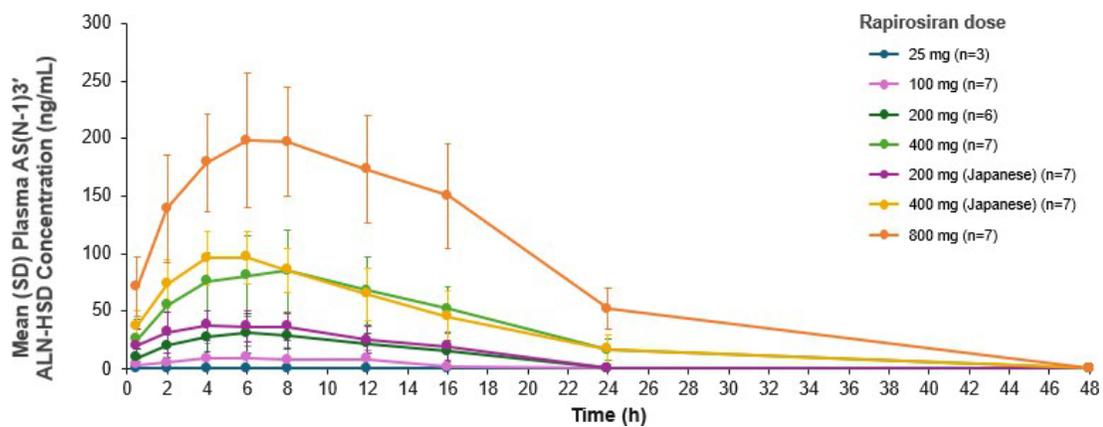
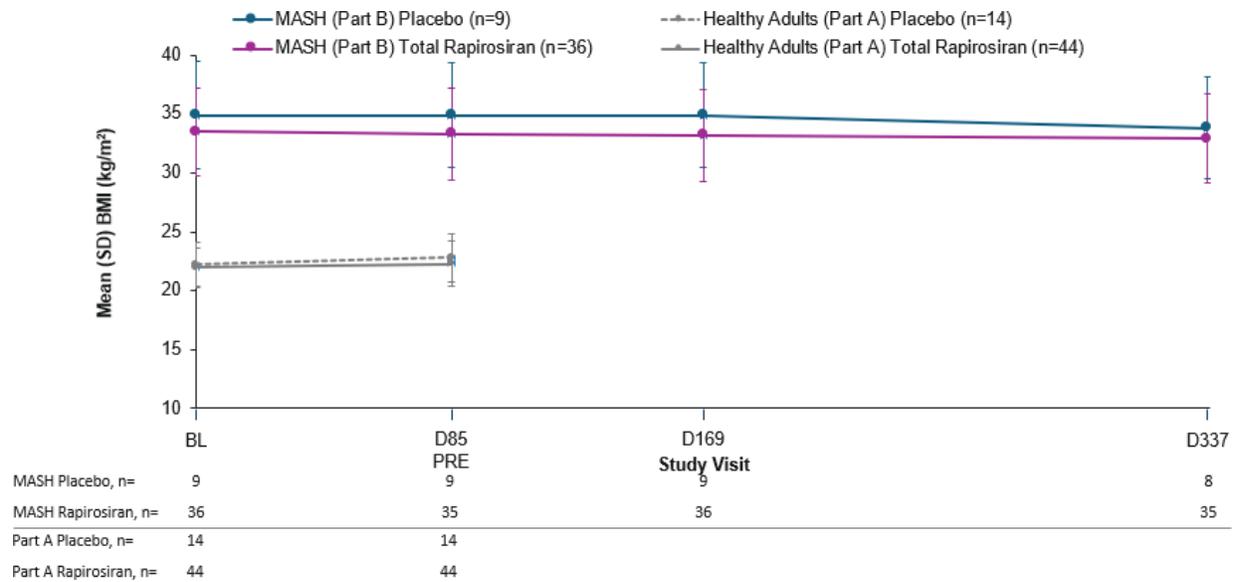
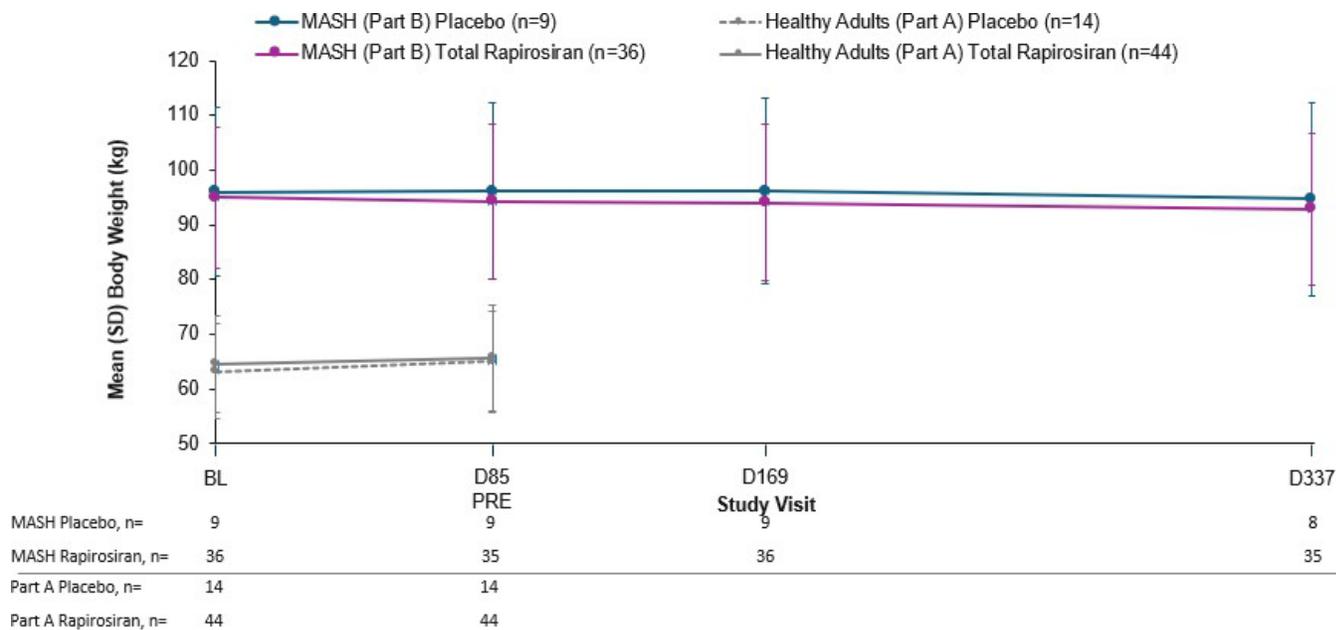


Fig. S3. BMI over time.



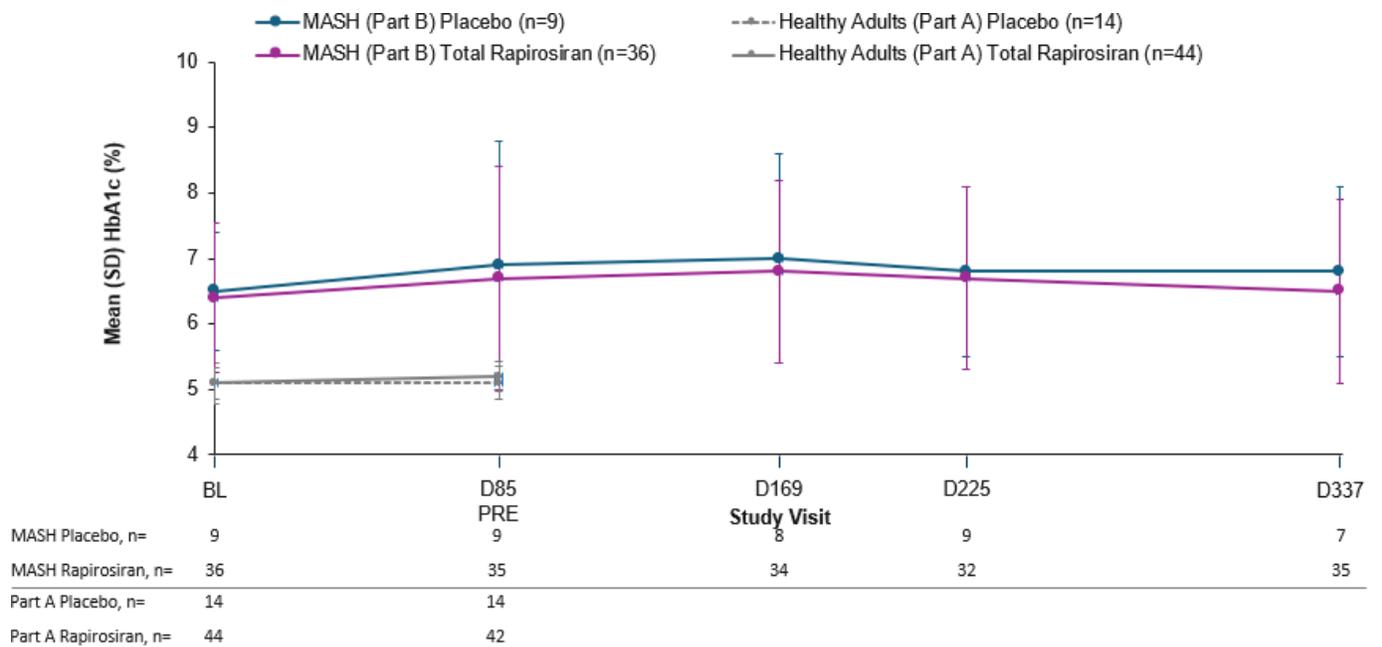
Part A participants (healthy adults) were assessed at baseline and Day 85 only. BL, baseline; BMI, body mass index; D, Day; MASH, metabolic dysfunction–associated steatohepatitis; PRE, within 7 days/pre-dose.

Fig. S4. Body weight over time.



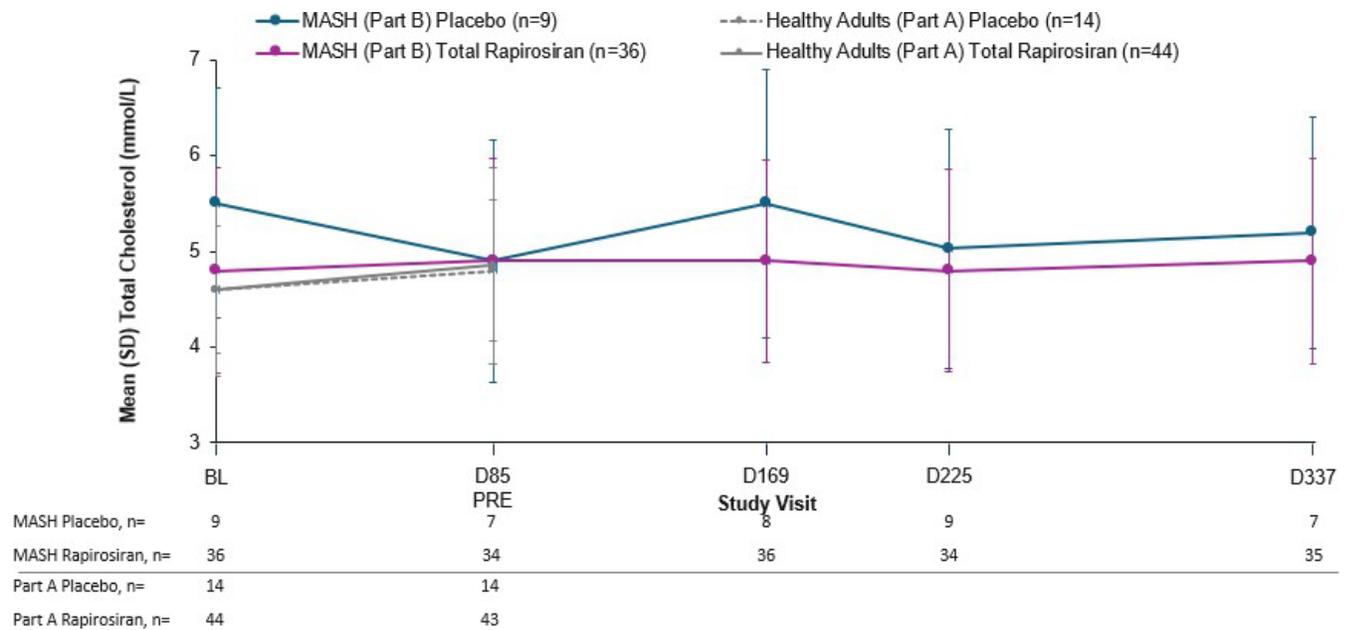
Part A participants (healthy adults) were assessed at baseline and Day 85 only. BL, baseline; D, Day; MASH, metabolic dysfunction–associated steatohepatitis; PRE, within 7 days/pre-dose.

Fig. S5. HbA1c over time.



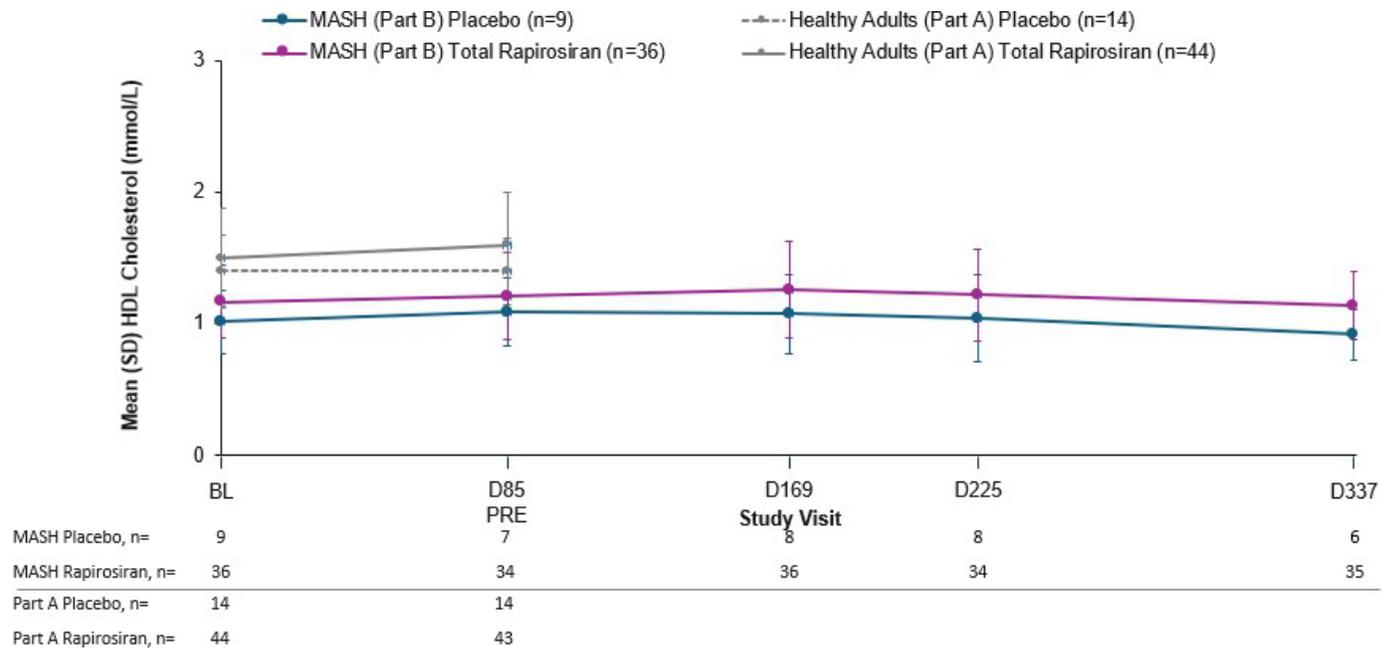
Part A participants (healthy adults) were assessed at baseline and Day 85 only. BL, baseline; D, Day; HbA1c, hemoglobin A1c; MASH, metabolic dysfunction–associated steatohepatitis; PRE, within 7 days/pre-dose.

Fig. S6. Total cholesterol over time.



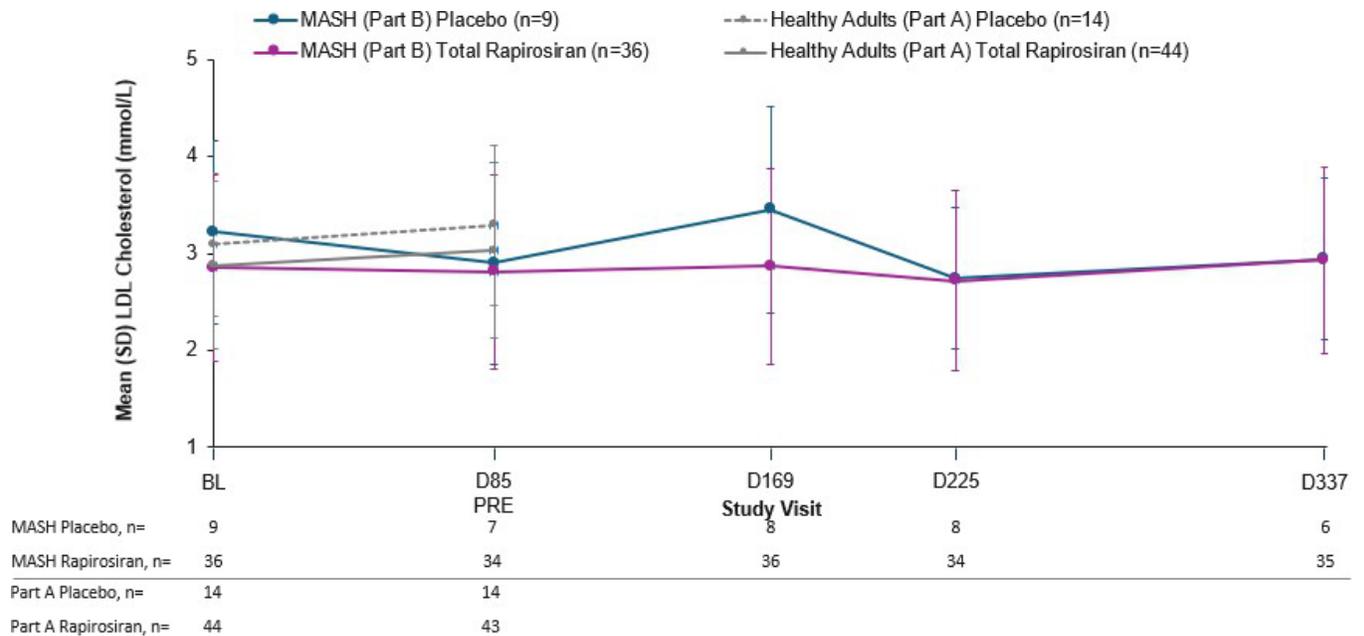
Part A participants (healthy adults) were assessed at baseline and Day 85 only. BL, baseline; D, Day; MASH, metabolic dysfunction–associated steatohepatitis; PRE, within 7 days/pre-dose.

Fig. S7. HDL cholesterol over time.

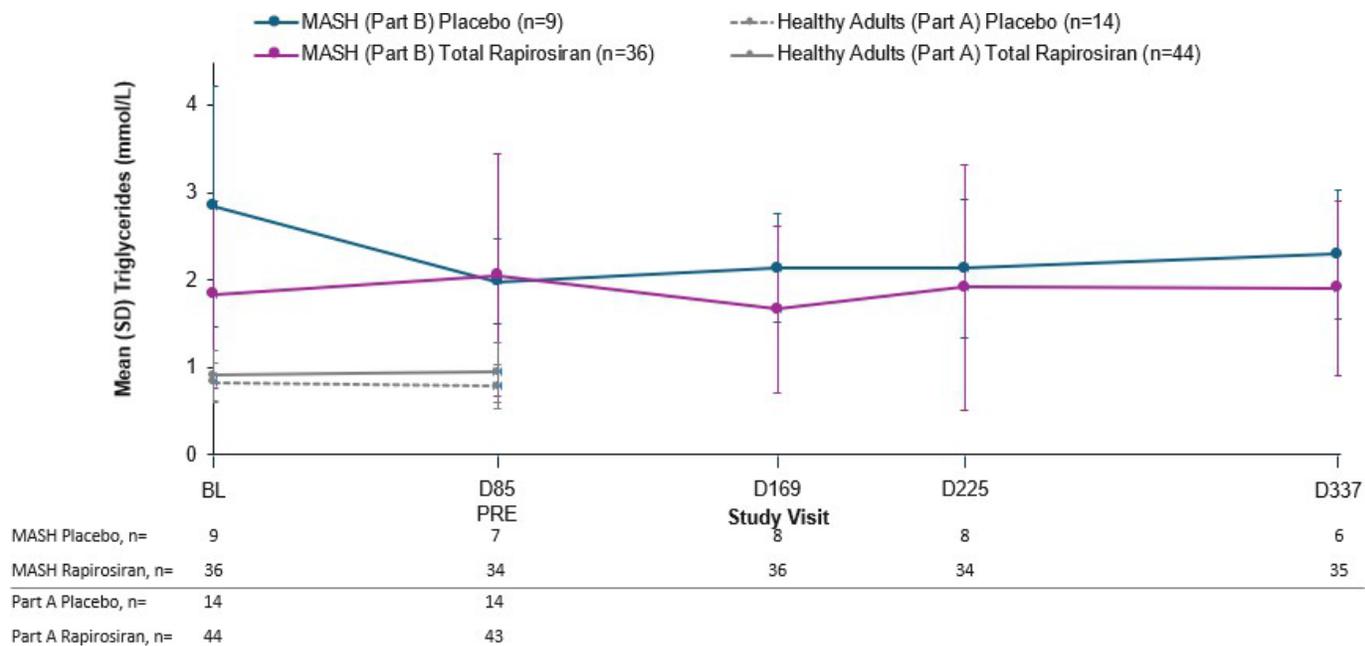


Part A participants (healthy adults) were assessed at baseline and Day 85 only. BL, baseline; D, Day; HDL, high-density lipoprotein; MASH, metabolic dysfunction–associated steatohepatitis; PRE, within 7 days/pre-dose.

Fig. S8. LDL cholesterol over time.



Part A participants (healthy adults) were assessed at baseline and Day 85 only. BL, baseline; D, Day; LDL, low-density lipoprotein; MASH, metabolic dysfunction-associated steatohepatitis; PRE, within 7 days/pre-dose.

Fig. S9. Triglycerides over time.

Part A participants (healthy adults) were assessed at baseline and Day 85 only. BL, baseline; D, Day; MASH, metabolic dysfunction–associated steatohepatitis; PRE, within 7 days/pre-dose.

Table S1. Genotype frequencies for *HSD17B13* and *PNPLA3* SNPs for all randomized patients in study Part B.

Allele	Placebo (n=9)	Rapirosiran (n=36)
<i>HSD17B13</i>, n (%)		
rs62305723:G		
Homozygous for risk alleles (G/G)	8 (89)	29 (81)
Heterozygous for risk allele (G/A)	1 (11)	1 (3)
Homozygous for protective alleles (A/A)	0	0
rs72613567:T		
Homozygous for risk alleles (T/T)	9 (100)	29 (81)
Heterozygous for risk allele (T/TA)	0	1 (3)
Homozygous for protective alleles (TA/TA)	0	0
rs80182459:GG		
Homozygous for risk alleles (GG/GG)	7 (78)	20 (56)
Heterozygous for risk allele (GG/G)	2 (22)	10 (28)
Homozygous for protective alleles (G/G)	0	0
<i>PNPLA3</i>, n (%)		
rs738409:G (I148M)		
Homozygous for risk alleles (G/G)	2 (22)	6 (17)
Heterozygous for risk allele (C/G)	3 (33)	10 (28)
Homozygous for protective alleles (C/C)	4 (44)	14 (39)

Table S2. Mean (%CV) Pharmacokinetic parameters in study Part A.^a

Parameter	25 mg (n=3)	100 mg (n=7)	200 mg (n=6)	200 mg, Japanese cohort (n=7)	400 mg (n=7)	400 mg, Japanese cohort (n=7)	800 mg (n=7)
Rapirosiran, plasma							
C _{max} , ng/mL	80.1 (19.2)	328.1 (62.2)	763.2 (38.4)	1016.0 (39.3)	1928.6 (24.4)	2142.9 (11.0)	4472.9 (24.7)
T _{max} , h ^b	6.02 (4.0, 8.0)	4.00 (2.0, 12.0)	5.01 (4.0, 8.0)	4.00 (0.5, 8.0)	6.07 (4.0, 16.0)	4.00 (2.0, 6.0)	6.00 (2.0, 12.1)
AUC _{last} , ng·h/mL	954.7 (21.4)	4379.6 (44.7)	10170.0 (30.1)	11761.9 (28.7)	27074.2 (22.1)	27886.3 (16.2)	68563.2 (23.5)
AUC _{inf} , ng·h/mL	1106.9 (—) ^c	4468.7 (47.5) ^d	10967.0 (26.4)	12229.5 (27.9)	28765.7 (24.1) ^d	30415.2 (15.2) ^d	74879.7 (21.2) ^e
t _{1/2} , h	5.4 (—) ^c	4.2 (51.7) ^d	5.6 (38.6)	4.3 (24.1)	5.7 (30.2) ^d	5.6 (36.7) ^d	5.5 (40.0) ^e

Rapirosiran, urine							
fe, %	16.7 (17.1)	18.7 (39.3)	22.9 (37.1)	34.8 (20.5)	31.0 (26.7)	36.5 (24.8)	32.6 (26.1) ^d
CL _R , L/h	3.2, 5.1 ^f	4.4 (20.8)	4.5 (20.7)	6.1 (18.7)	4.9 (27.1)	5.4 (24.3)	4.3 (37.8) ^d
AS(N-1)3' ALN-HSD, plasma							
C _{max} , ng/mL	NC	11.7 (113.4)	32.2 (52.6)	42.1 (34.1)	91.4 (31.7)	101.8 (18.5)	216.1 (23.1)
T _{max} , h ^b	NC	5.00 (2.0, 12.0) ^g	6.00 (4.0, 8.0)	8.00 (0.5, 8.0)	6.07 (4.0, 16.0)	4.00 (4.0, 6.0)	6.03 (6.0, 12.1)
AUC _{inf} , ng·h/mL	NC	NC	NC	590.2, 724.2 ^f	1439.4 (32.1) ^e	1701.0 (17.6) ^g	4082.0 (24.9) ^g
t _½ , h	NC	NC	NC	4.5, 7.1 ^f	6.7 (21.1) ^e	5.5 (28.2) ^g	6.8 (27.7) ^g
MR _{AUClast}	NC	0.03 (27.4) ^g	0.03 (15.0)	0.04 (6.1)	0.05 (10.6)	0.05 (15.4)	0.05 (7.7)

MR _{Cmax}	NC	0.03 (94.4)	0.04 (14.0)	0.04 (10.8)	0.05 (9.1)	0.05 (11.2)	0.05 (9.8)
AS(N-1)3' ALN-HSD, Urine							
fe, %	0.6 (14.0)	0.8 (43.9)	0.9 (37.5)	1.6 (26.7)	1.5 (29.0)	1.8 (24.9)	1.7 (22.3)

^aSome metabolite PK parameters could not be calculated, or were calculated with limited data, due to metabolite concentrations in plasma being below the lower limit of quantitation (<10.0 ng/mL). AUC_{inf} and t_{1/2} are not reported if Lambda z (λ_z) is not estimable. Estimation of λ_z requires a minimum of 3 quantifiable data points on the terminal log-linear phase, not including C_{max}. ^bMedian (minimum, maximum) is presented for T_{max}.

^cn=1, individual participants' data are reported. ^dn=6. ^en=5. ^fn=2, individual PK parameters are reported. ^gn=4.

Rapirosiran, GalNAc-siRNA conjugate that targets human *HSD17B13* mRNA; AS(N-1)3' ALN-HSD, double-stranded metabolite of rapirosiran with loss of 1 nucleotide from the 3' terminus of the antisense strand; AUC, area under the concentration-time curve; AUC_{inf}, AUC from time of dosing extrapolated to infinity; AUC_{last}, AUC from time of dosing to the last measurable concentration; C_{max}, maximum steady-state plasma drug concentration; CL_R, renal clearance; CV, coefficient of variation; fe, fraction excreted; GalNAc, *N*-acetylgalactosamine; MR_{AUClast}, metabolite-to-parent ratio for AUC_{last}; MR_{Cmax}, metabolite-to-parent ratio for C_{max}; N, number of patients; NC, not calculated; PK, pharmacokinetics; siRNA, small interfering mRNA; t_{1/2}, terminal half-life; T_{max}, time to reach peak concentration.