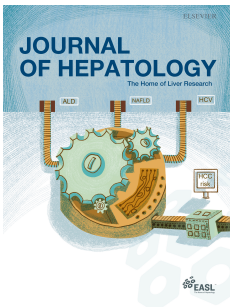

Phase 1 randomized double-blind study of an RNA interference therapeutic targeting *HSD17B13* for metabolic dysfunction–associated steatohepatitis

Arun J. Sanyal, Jorg Taubel, Prajakta Badri, Sarah Bond, Nune Makarova, Weizhi Zhao, Swati Duggal, Farshad Kajbaf, Benjamin A. Olenchock, John M. Gansner

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e 1 randomized double-blind study of an RNA interference therapeutic targeting *HSD17B13* for metabolic dysfunction–associated steatohepatitis

Background

Loss of function in *HSD17B13* is associated with reduced risk of chronic liver disease

Rapirosiran, an investigational RNA interference therapeutic, targets liver-expressed *HSD17B13* mRNA

Methods

Participants

- 18-65 years old
- **Part A**: Healthy adults (N=58)
- **Part B**: MASH (N=46)

Treatments

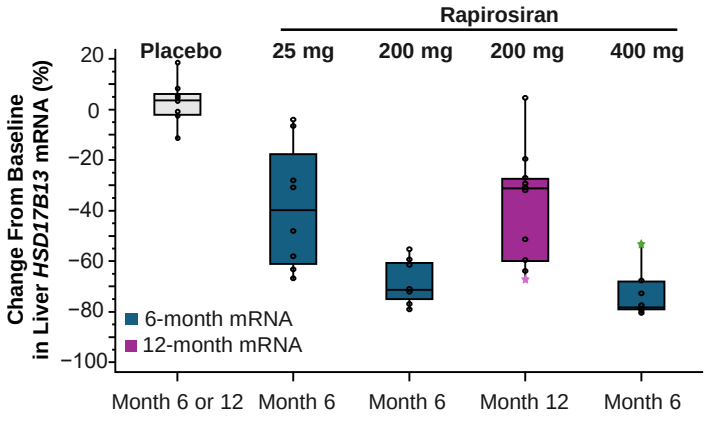
- Rapirosiran
- Placebo

Endpoints

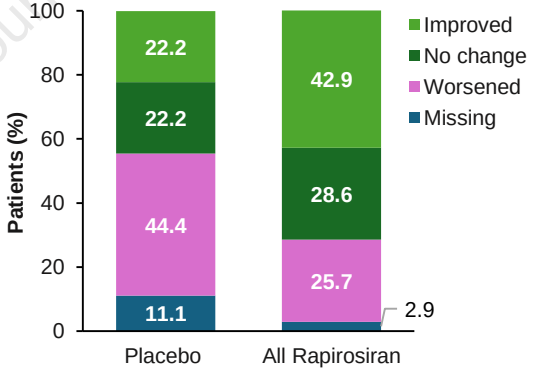
- **Part A:** AEs, PK
- **Part B:** AEs, change in liver *HSD17B13* mRNA, NAFLD Activity Score

Results

Percentage Change From Baseline in *HSD17B13* mRNA



Change From Baseline in NAFLD Activity Total Score



AEs in ≥10% of those treated with rapirosiran

Part A (healthy adults): Injection-site reactions (11%), all mild and transient

Part B (MASH): COVID-19 (14%), non–treatment-related

No evidence of drug-induced liver injury in any participants

Phase 1 randomized double-blind study of an RNA interference therapeutic targeting *HSD17B13* for metabolic dysfunction–associated steatohepatitis

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Short title: Phase 1 Trial of RNAi Rapirosiran in MASH

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Keywords: [provide 3–12 keywords from MeSH list] RNAi therapeutics; non-alcoholic fatty liver disease; metabolic dysfunction–associated steatohepatitis; liver diseases; *HSD17B13*

Word count: 5880/6000; Abstract: 270/275

Figures: 6 **Tables:** 2 (8/8 total)

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, Akero 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, Akero, 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.

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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

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

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.

ABSTRACT

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 1 study, we evaluated safety, tolerability, pharmacokinetics, and pharmacodynamics of rapirosiran, an investigational, *N*-acetylgalactosamine (GalNAc)-conjugated small interfering RNA targeting liver-expressed mRNA for *HSD17B13*.

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 rapirosiran 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). Rapirosiran plasma and urine pharmacokinetics and change from baseline of liver *HSD17B13* mRNA were secondary endpoints.

Results

In Part A, the only AE occurring in ≥10% of rapirosiran–treated subjects was injection-site reaction (11%); all occurrences were mild and transient. There were no treatment-related serious AEs (SAEs). Plasma concentrations of rapirosiran declined rapidly by 24

hours post-dose. Across doses, rapirosiran showed 17%–37% excretion in urine. In Part B, the only AE occurring in ≥10% of rapirosiran–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. Rapirosiran was associated with dose-dependent reduction of liver *HSD17B13* mRNA in Part B, with a median reduction of 78% at 6 months in the highest-dose (400 mg) group.

Conclusions

Rapirosiran exhibited an encouraging safety and tolerability profile, with robust reduction in liver *HSD17B13* mRNA expression.

IMPACT AND IMPLICATIONS

Metabolic dysfunction–associated steatohepatitis (MASH) is a prevalent chronic liver disease with a high burden of disease. The hydroxysteroid 17-beta dehydrogenase 13 gene (*HSD17B13*) is implicated in the pathogenesis of MASH. Rapirosiran offers a novel mechanism to treat MASH by directly reducing hepatic *HSD17B13* expression. In this Phase 1 study, rapirosiran demonstrated an encouraging safety and tolerability profile and resulted in 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.

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

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 United States, 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 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 1 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 United Kingdom (UK) between October 2020 and September 2021. Part B was conducted at 13 clinical sites across 5 countries (the United States, 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 body mass index (BMI) of 18 to 28 kg/m^2, had normal aspartate

aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin levels, and had an estimated glomerular filtration rate of ≥90 mL/min/1.73 m^2. 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/m^2, and had a diagnosis of MASH documented in their medical history or a clinical suspicion for MASH based on specific criteria (ie, 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 activity score (NAS) of 3 or more according to NASH Clinical Research Network criteria, including at least 1 point for each of the three key NASH histological features (ie, 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 m^2, ALT level >5 times the upper limit of normal (ULN), international normalized ratio >1.2, platelet count <140 × 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 hour pre-dosing and at 30 minutes and 2, 4, 6, 8, 12, 24, and 48 hours after rapirosiran administration. Blood samples were evaluated for antidrug antibodies (ADA) against rapirosiran. Urine samples were collected from each subject after rapirosiran administration on Day 1, with pooled collections obtained over intervals of 0–6, 6–12, and 12–24 hours. 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 rapirosiran and its metabolite duplex concentration 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 RNA*later* tissue storage reagent. Quantification of *HSD17B13* was performed by reverse transcription polymerase chain reaction. 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 (ie, 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 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 cutoff 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 (AUC_{inf}), terminal phase elimination rate constant (λz), half-life ($t_{½}$), renal clearance (Cl_{ren}), and percentage excreted in urine (fe %). 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 $y=\alpha \times Dose^{\beta}$, 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 (CI) 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/m^2, respectively). Mean 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 (Supplementary Table 1).

Adverse events 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 >3 × ULN if normal at baseline or >3 × 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% or more 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 SAE 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 >3 times the ULN and an AST level >5 times 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 four patients (11%) 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. There were two AEs deemed treatment-related in two patients (22%) treated with placebo: abdominal pain and nausea. In Part B, two patients (6%) 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 Supplementary Table 2. Rapirosiran was absorbed with a median T_{max} of 4.0 to 6.1 hours. Plasma

concentrations of rapirosiran declined rapidly and were undetectable by 48 hours in most subjects (Supplementary Fig. 1), with a mean terminal half-life ($t_{1/2}$) ranging between 4.2 and 5.7 hours. 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 hours, and the levels in plasma were undetectable by 24 hours in most subjects (Supplementary Fig. 2). The $t_{1/2}$ of AS(N-1)3′ ALN-HSD (mean value 5.5 to 6.8 hours) was similar to that of rapirosiran. The metabolite-to-parent ratios for the C_{max} (MR_{Cmax}) and area under the concentration-time curve AUC_{last} ($MR_{AUClast}$) were low; both MR_{Cmax} and $MR_{AUClast}$ 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% CIs) 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 subjects. 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 change in *HSD17B13* mRNA at Month 6 relative to baseline was −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 (Fig. 4 and Fig. 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 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 rapirosiran 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 rapirosiran compared with placebo.

Anthropometric and metabolic measurements over time (BMI, weight, hemoglobin A1c, lipid parameters) are presented in Supplemental Figs. 3-9.

DISCUSSION

The purpose of this study was to characterize the PK, PD, and safety and tolerability profile of rapirosiran to support dose selection of rapirosiran for Phase 2 clinical trials. Rapirosiran 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 1 study, rapirosiran 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 rapirosiran, C_{max} was reached by 4 to 6 hours post-dose. The rapid decline in plasma concentrations of rapirosiran and its metabolite to lower limits of quantification by 48 hours 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 hours 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 subjects.[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 subjects. 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 impairment is not anticipated to have a major impact on the PK of rapirosiran and AS(N-1)3′ ALN-HSD. The incidence of treatment-emergent

ADAs was low in those treated with rapirosiran, 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 rapirosiran 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 $t_{½}$ of rapirosiran and its metabolite. This indicates that rapirosiran has a long $t_{½}$ 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]

Rapirosiran 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 rapirosiran 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 (eg, most taken only 3 months after the last dose), the heterogenous MASH population inclusive of fibrosis stage F0-F3 with a NAS ≥3, and the inclusion of participants

agnostic of genotype (ie, not restricting to *HSD17B13* risk allele homozygotes). The impact of *HSD17B13* mRNA liver knockdown on liver histology will be more definitively answered by the ongoing Phase 2 study (NCT05519475), evaluating rapirosiran 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 (ie, homozygous for protective allele carriers who might be unlikely to derive benefit from targeting *HSD17B13*). However, multiple subjects 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 1 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, rapirosiran exhibited an encouraging safety and tolerability profile in the Phase 1 ALN-HSD-001 study, with robust reduction in liver *HSD17B13* mRNA expression, supporting further clinical development.

ABBREVIATIONS

AE, adverse event

ALT, alanine aminotransferase

AST, aspartate aminotransferase

BMI, body mass index

GalNAc, *N*-acetylgalactosamine

GWAS, genome-wide association study

HSD17B13, hydroxysteroid 17-beta dehydrogenase 13

MASH, metabolic dysfunction–associated steatohepatitis

NAS, NASH activity score

NASH, non-alcoholic steatohepatitis

PD, pharmacodynamic(s)

PK, pharmacokinetic(s)

RNAi, RNA interference

ULN, upper limit of normal

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TABLES

Table 1. Demographics and baseline characteristics.

	Placebo (n=14)	**Rapirosiran (n=44)**
Part A: Healthy participants		
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)
	Placebo (n=9)	**Rapirosiran (n=36)[c]**
Part B: Patients with MASH		
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)

[a]ALT normal range: 10–35 U/L (females), 10–50 U/L (males). [b]AST normal range: 0–31 U/L (females), 0–37 U/L (males). [c]One patient with hepatitis E withdrew from the study prior to receiving the first dose due to increased values on liver function tests. [d]ALT normal range: ≤33 U/L (females), ≤41 U/L (males). [e]AST normal range: ≤31 U/L (females), ≤37 U/L (males).

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

Table 2. Treatment-emergent adverse events.

	Placebo (n=14)	**Rapirosiran (n=44)**
Part A: Healthy participants[a]		
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)
	Placebo (n=9)	**Rapirosiran (n=36)[f]**
Part B: Patients with MASH		
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,j]
TEAE leading to study drug discontinuation	0 (0)	1 (3)[i]
TEAE leading to study withdrawal	0 (0)	0 (0)
Death[e]	0 (0)	0 (0)

All data reported are n (%).

[a]Safety data reported over the 3-month double-blind period. [b]TEAE 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. [c]Includes bruising, blanching, and blanched skin at injection site. [d]AEs 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. [e]All deaths are included regardless of whether they were treatment-emergent. [f]One patient with hepatitis E withdrew from the study prior to receiving the first dose due to increased values on liver function tests. [g]One patient had appendicitis. [h]One patient had appendicitis, and 1 patient had a tooth infection. [i]One patient had increased liver enzyme levels (due to hepatitis E). [j]None of the ISR cases met the criteria for clinical interest.

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

FIGURE LEGENDS

Fig. 1. Study design. [a]Cohorts were enrolled sequentially. [b]Includes a dedicated Japanese cohort. BMI, body mass index; ECG, electrocardiogram; MASH, metabolic dysfunction–associated steatohepatitis; NAS, NASH activity score; NASH, non-alcoholic steatohepatitis; NASH CRN, NASH Clinical Research Network; Q12W, every 12 weeks; QTcF, Fridericia-corrected QT interval; SC, subcutaneous.

Fig. 2. Disposition of healthy participants (Part A) and patients with metabolic dysfunction–associated steatohepatitis (MASH; Part B). [a]One patient with hepatitis E withdrew from the study due to mildly increased values on liver function tests.

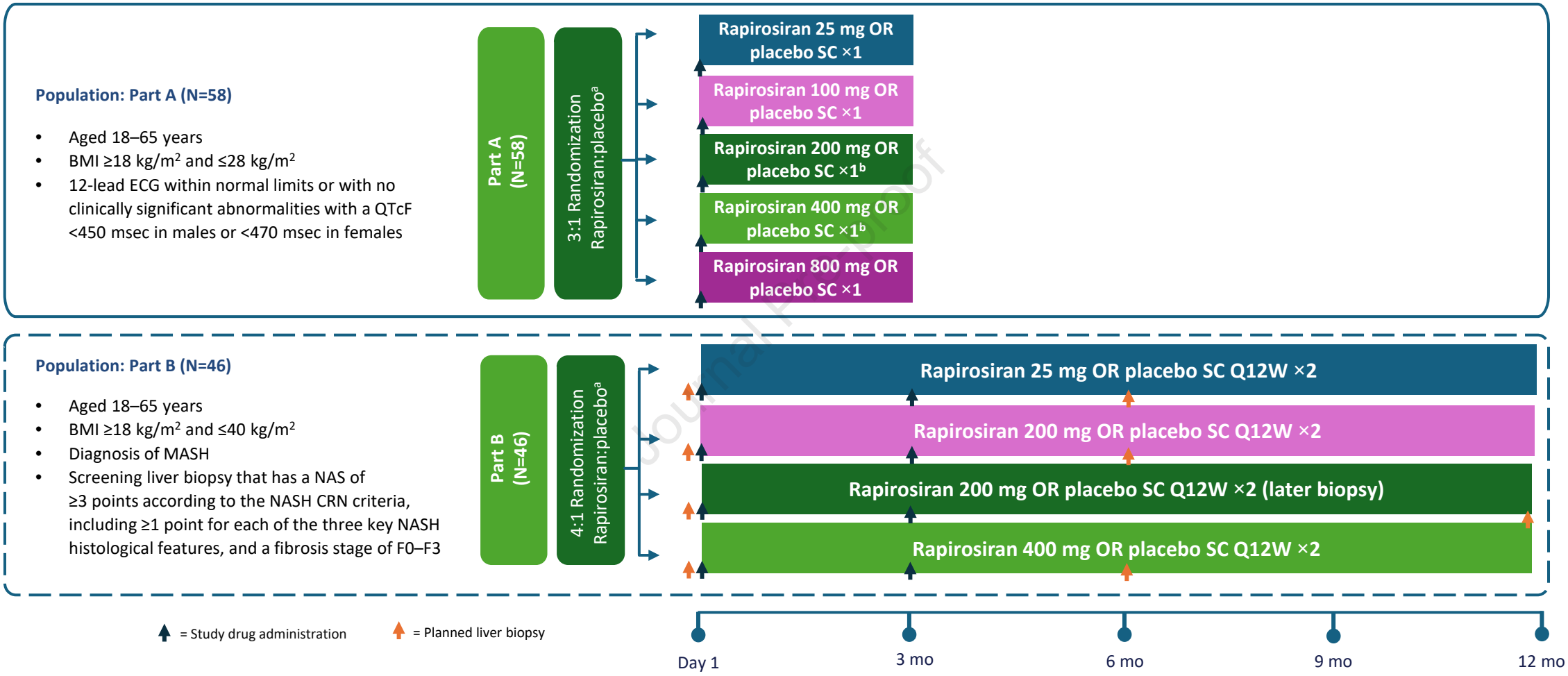
Fig. 3. *HSD17B13* mRNA percentage change in liver biopsies at Month 6 and Month 12 in patients with metabolic dysfunction–associated steatohepatitis. (A) Box plot, (B) Column plot. No patients had homozygous protective alleles for any of the 3 variants of *HSD17B13.* 12-month mRNA was not evaluated in the rapirosiran 25 mg or 400 mg dose groups. Boxplot: The pink star (rapirosiran, 200 mg, Month 12) indicates 1 patient who had liver biopsy at Month 6 instead of Month 12, and the green star (rapirosiran, 400 mg, Month 6) indicates 1 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.

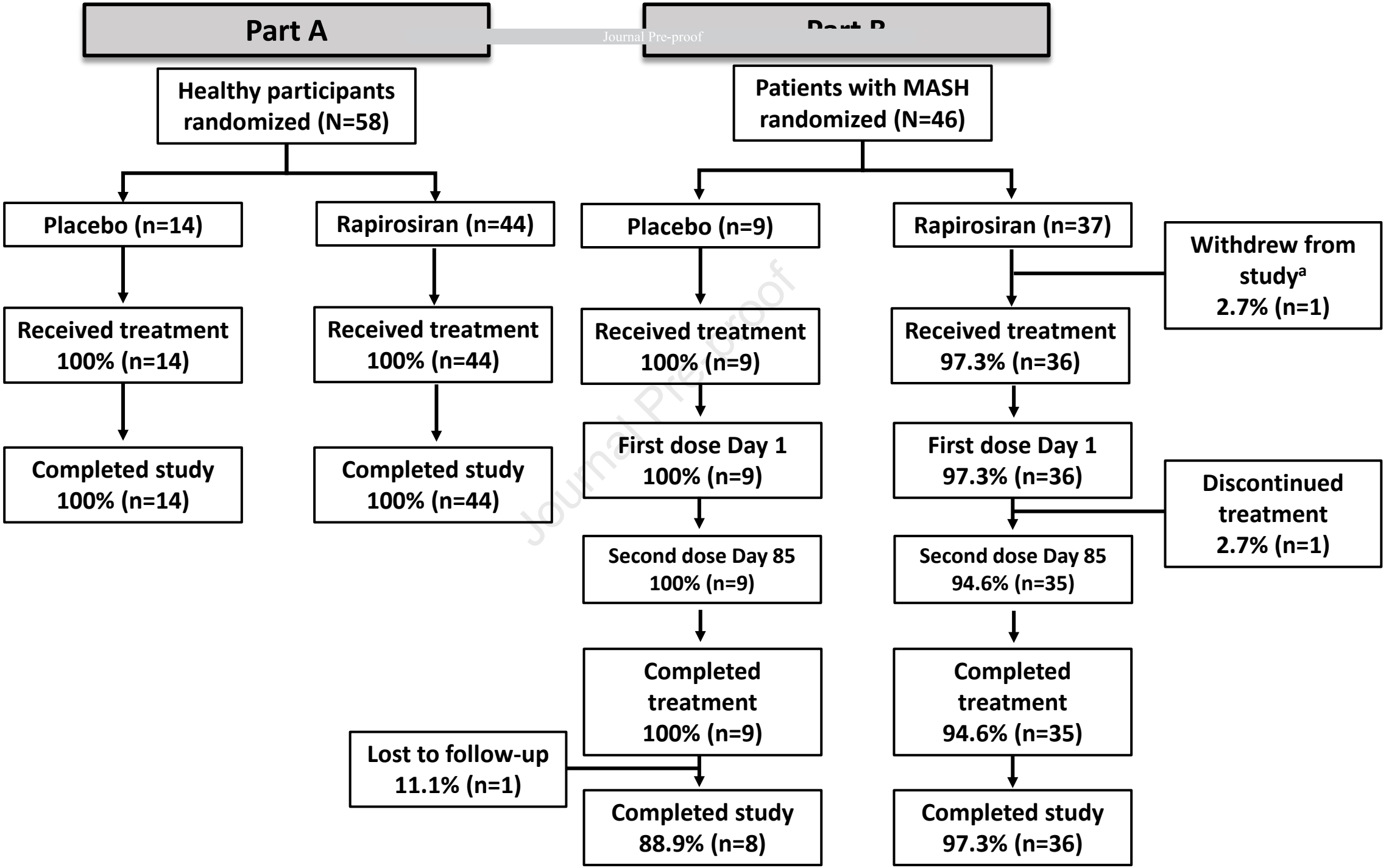
Fig. 4. ALT levels over time in patients with metabolic dysfunction–associated steatohepatitis. (A) Mean (SEM) actual values. (B) Mean (SEM) percentage change

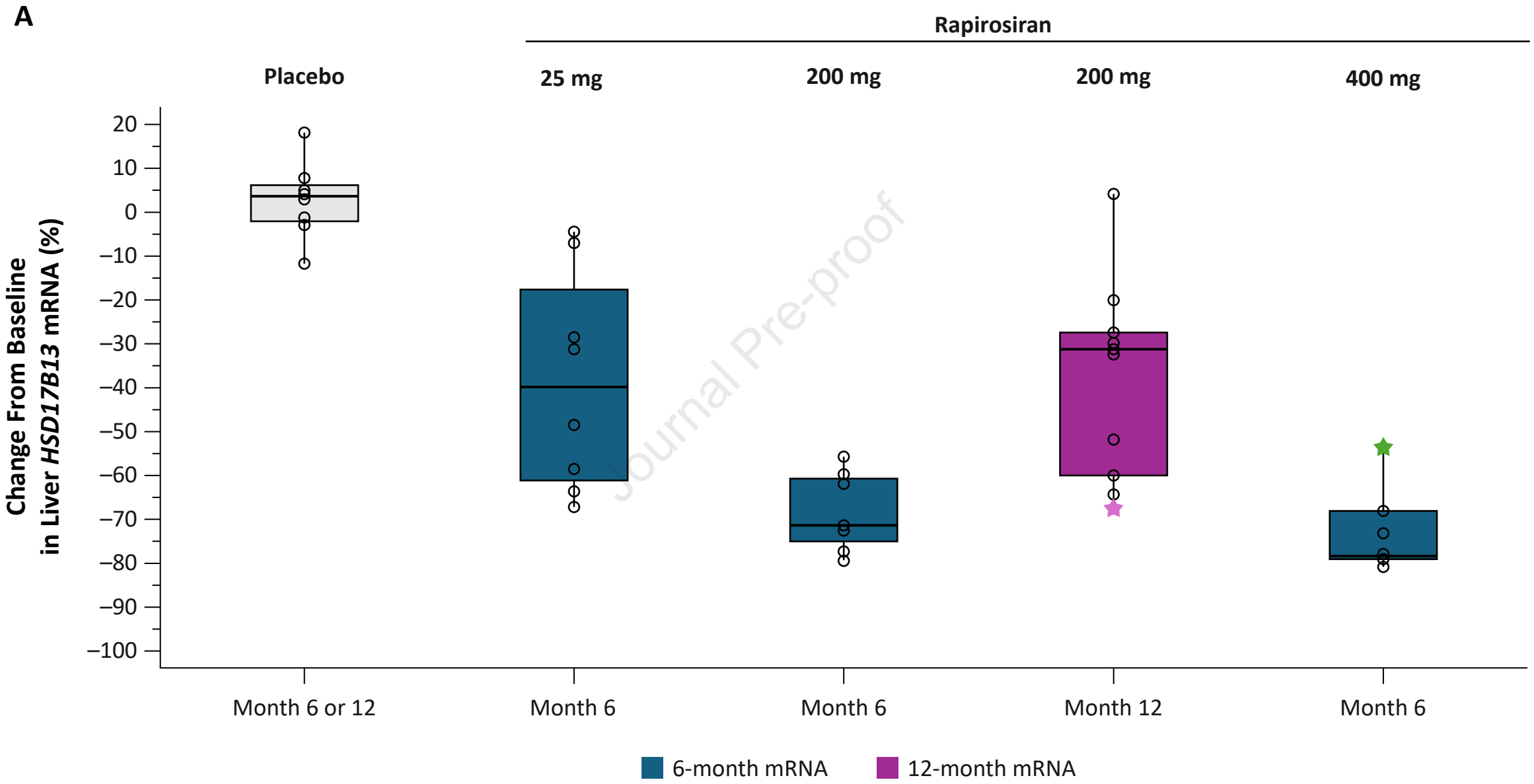
from baseline. ALT, alanine aminotransferase; BL, baseline; D, Day; W-7/PRE, within 7 days/pre-dose.

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.

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. [a]Six-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 1 patient who underwent liver biopsy at Month 6 instead of Month 12. [b]Subcomponent of NAFLD total activity score. [c]Second biopsy visit was cancelled in 1 patient out of 36 in the rapirosiran group. Worsened: change from baseline >0. Improved: change from baseline <0. NAFLD, non-alcoholic fatty liver disease.







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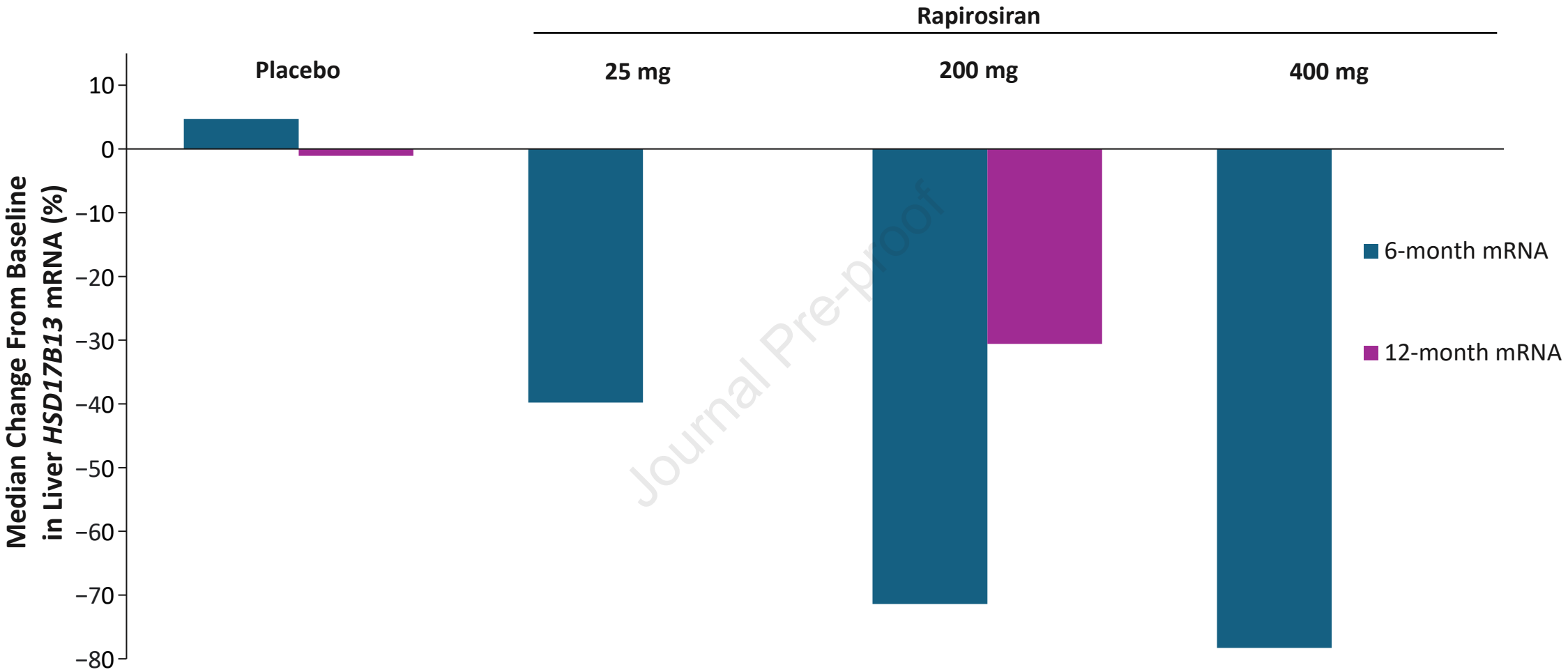
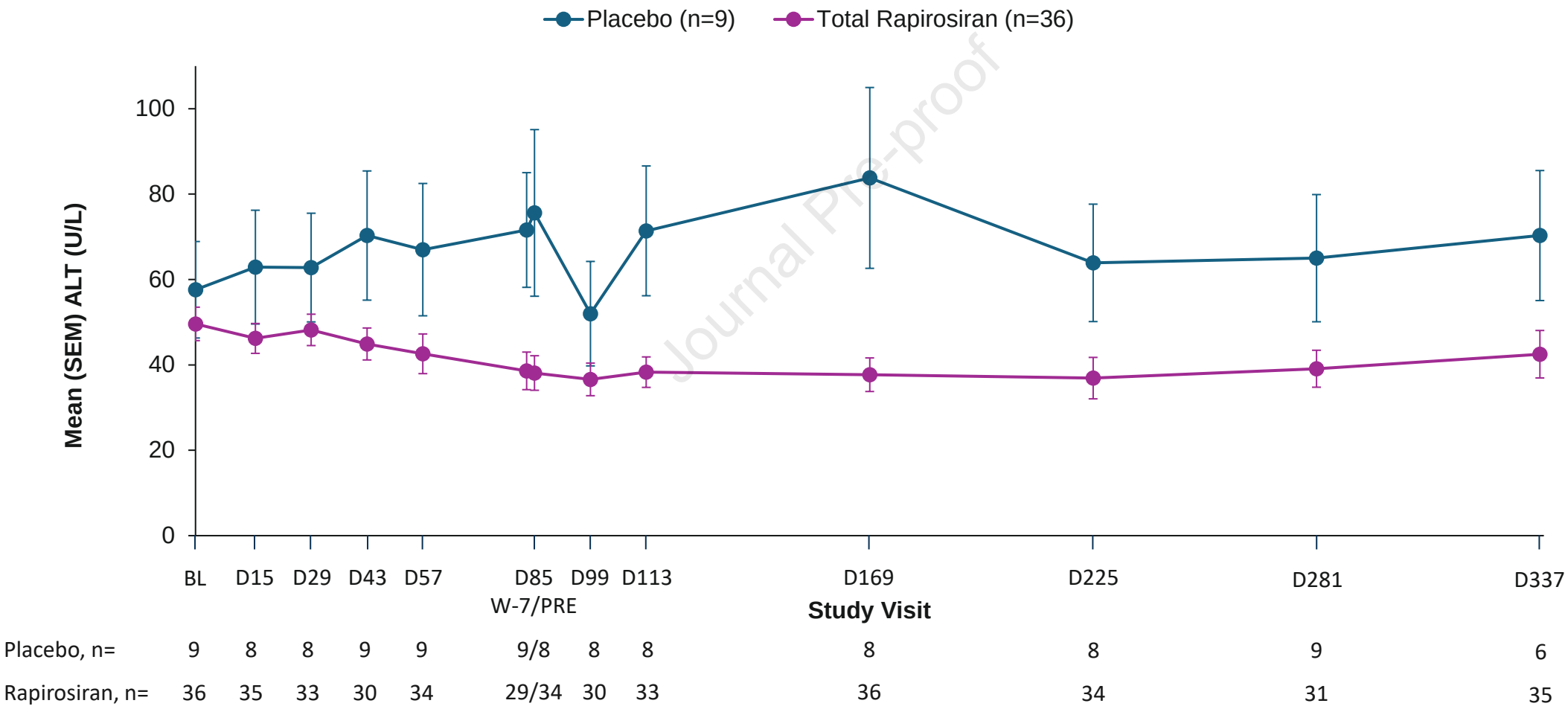


6-month mRNA, n=	5	8	9	7
12-month mRNA, n=	3	0	10	0

A



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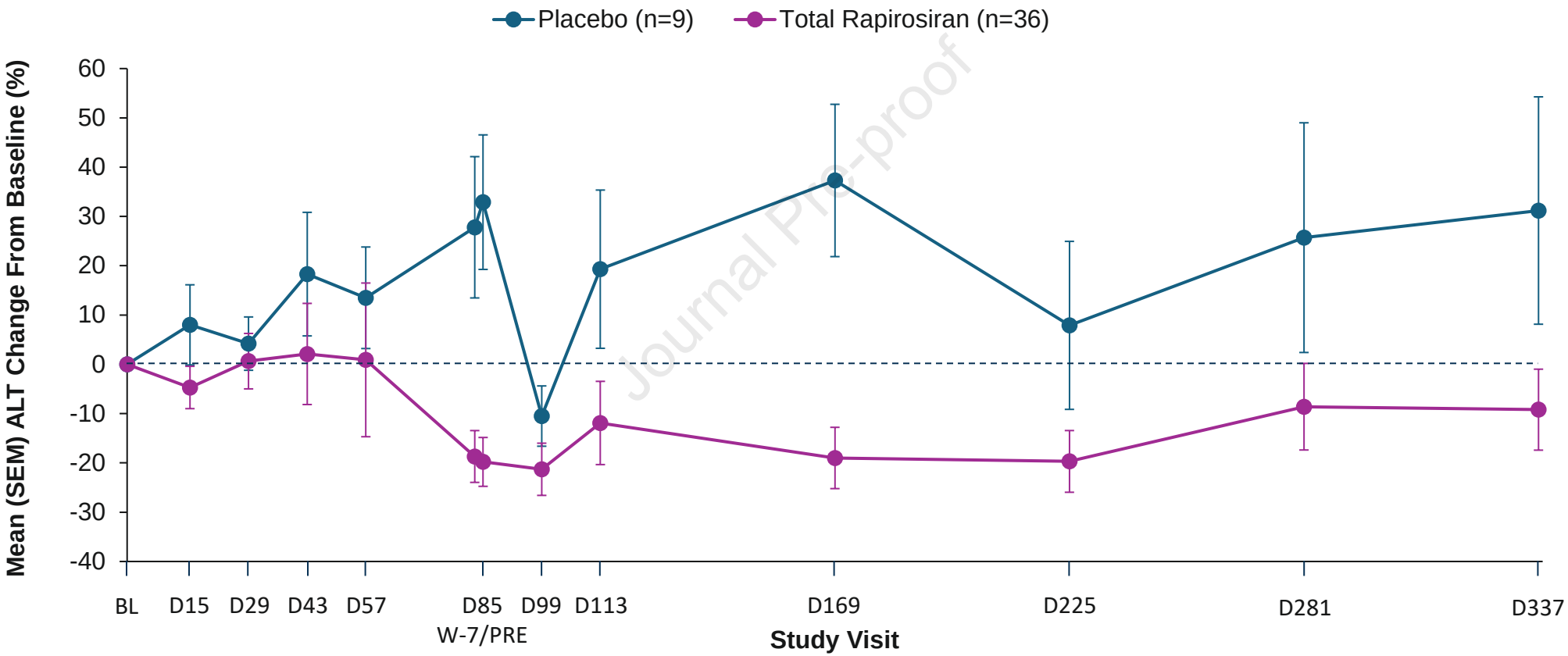

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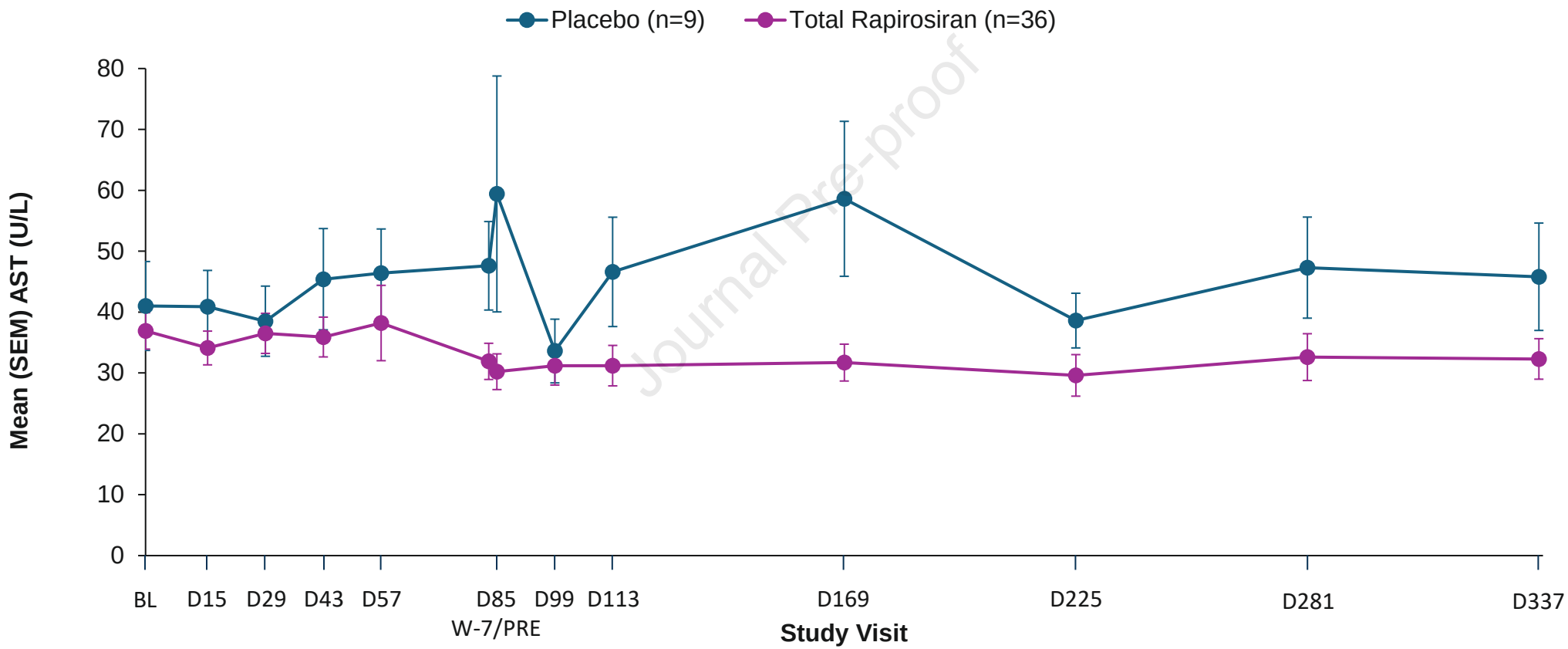

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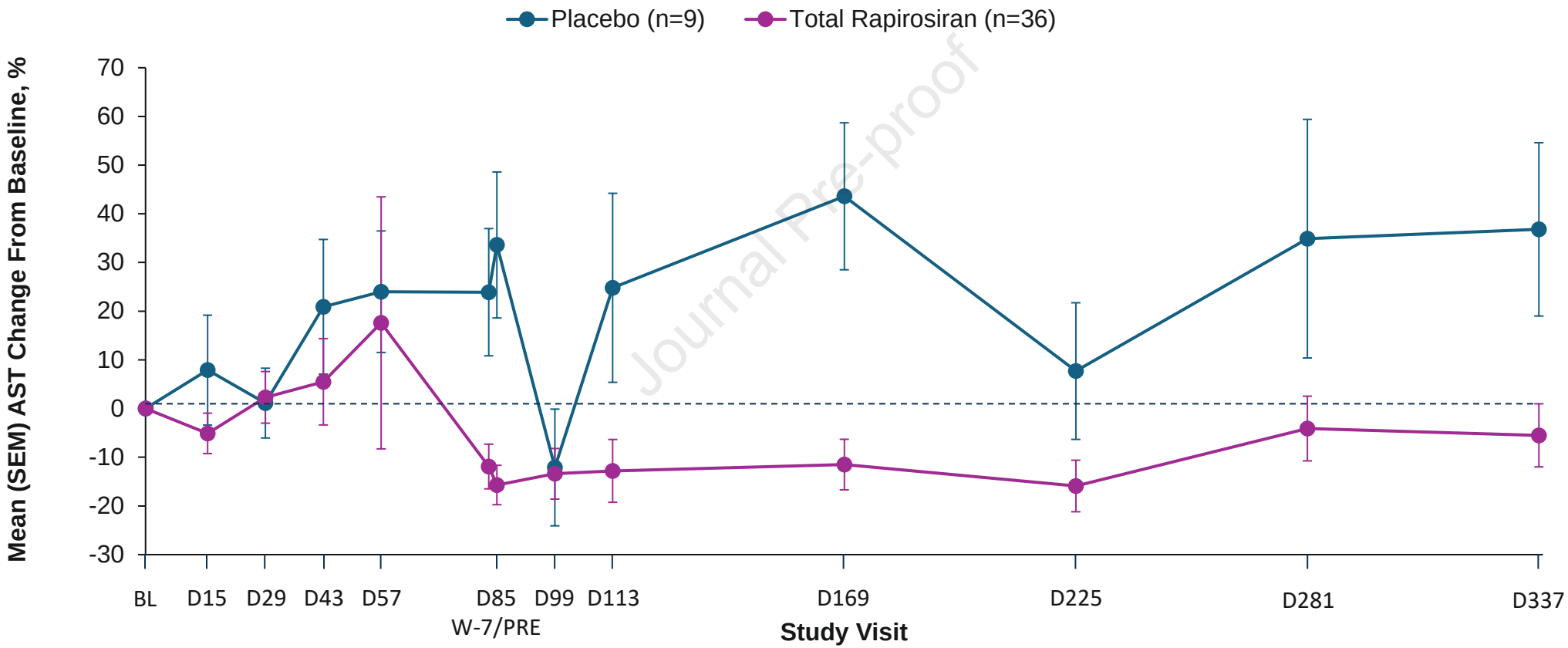

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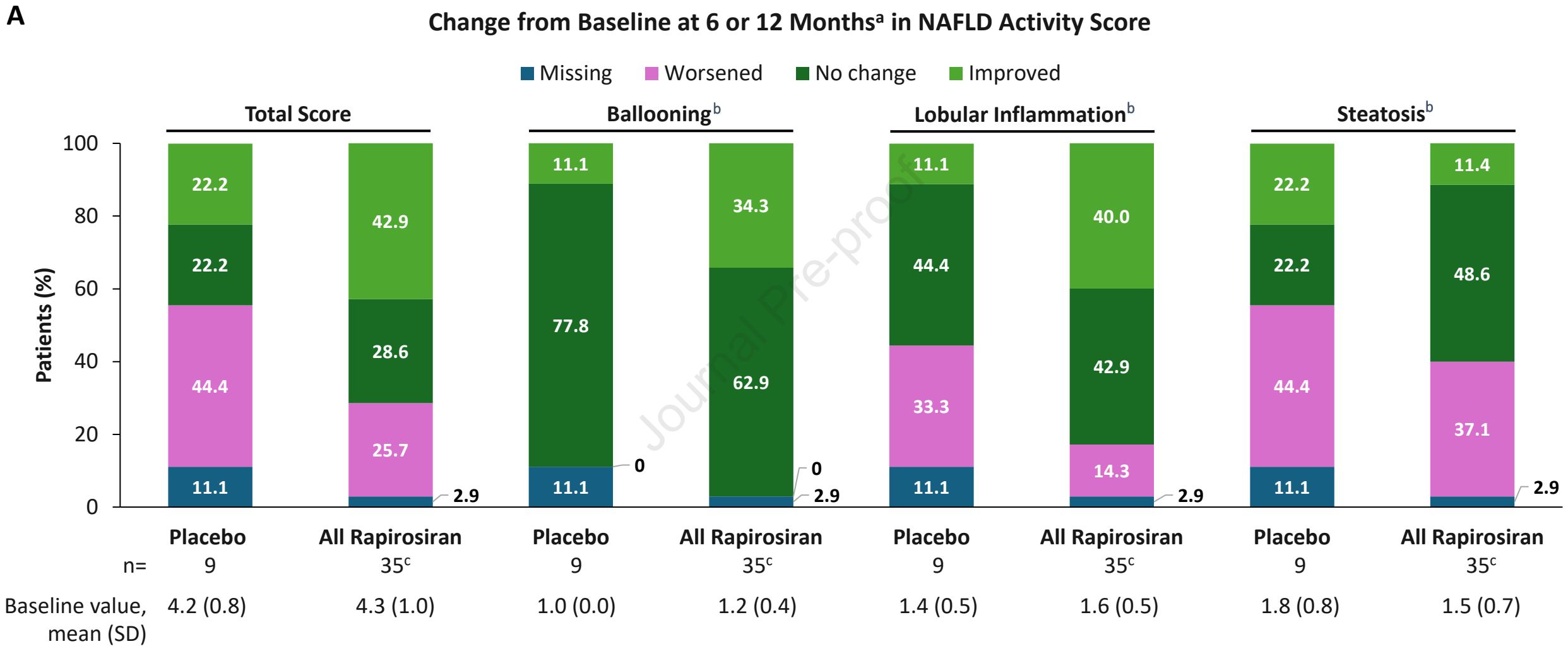
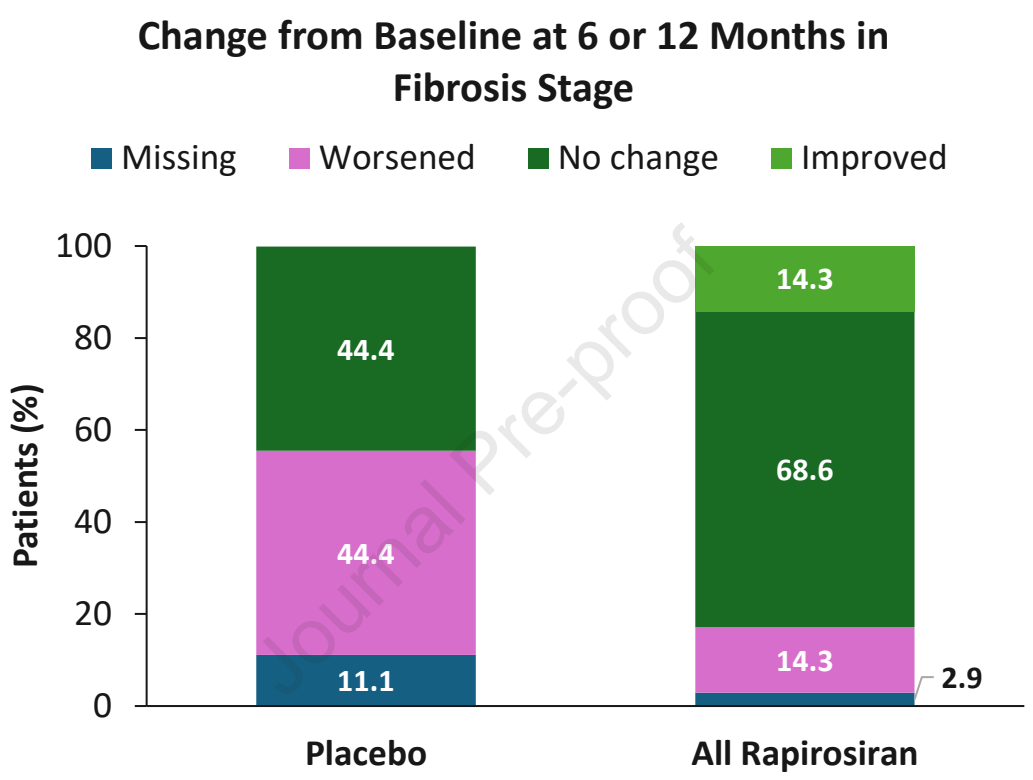


[a]12-month biopsies were only available in one of the two 200 mg cohorts. [b]Subcomponent of NAFLD total activity score. [c]Second biopsy visit was cancelled in one patient out of 36 in the rapirosiran group.

B



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