Phase 1 Study of the RNA Interference Therapeutic ALN-HSD in Healthy Adults and Patients with Nonalcoholic Steatohepatitis

Arun J. Sanyal[1], Jorg Taubel[2], Prajakta Badri[3], Sarah Bond[3], Nune Makarova[3], Weizhi Zhao[3], Satyajit Karnik[4], Farshad Kajbaf[3], Benjamin Olenchock[4], Joshua R. Friedman[3*], **John M. Gansner[3]**

[1]Division of Gastroenterology, Hepatology and Nutrition, Virginia Commonwealth University, Richmond, VA, USA
[2]Richmond Pharmacology and St. George's University of London, London, UK
[3]Alnylam Pharmaceuticals, Cambridge, MA, USA
[4]Regeneron Pharmaceuticals, Tarrytown, NY, USA

*Former employee of Alnylam Pharmaceuticals, current employee of Spark Therapeutics

Co-funded by Alnylam Pharmaceuticals and Regeneron Pharmaceuticals, Inc.

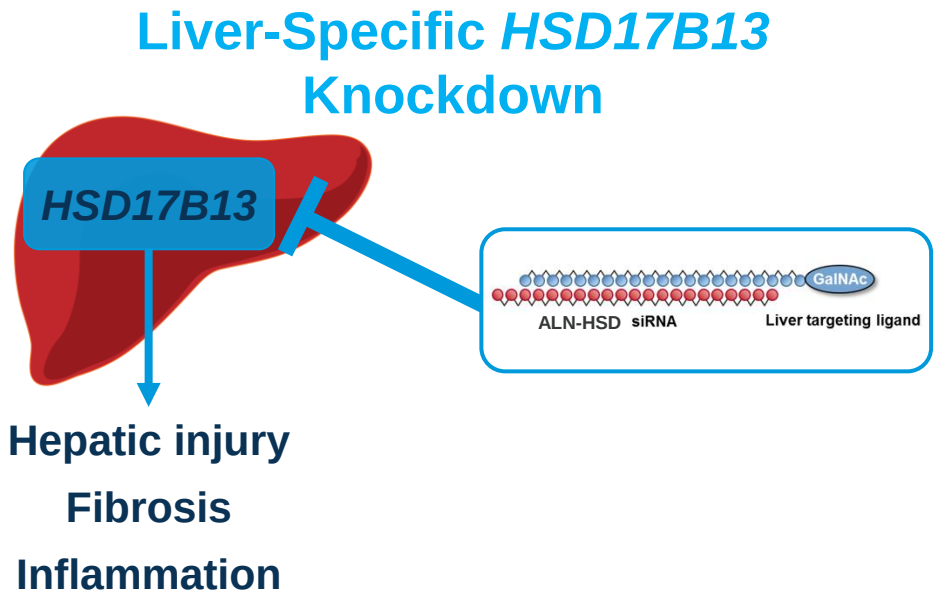
21–24 June 2023 || European Association for the Study of the Liver (EASL) Congress 2023

Introduction

- Nonalcoholic steatohepatitis (NASH) is a prevalent chronic liver disease that can lead to progressive fibrosis, cirrhosis, and hepatocellular carcinoma[1,2]
  - There are currently no approved pharmacological treatments for NASH, despite its rising burden and increasing prevalence[2]

- Genome-wide association studies have identified loss-of-function variants in the hydroxysteroid 17β dehydrogenase 13 (*HSD17B13*) gene that are associated with a reduced risk of chronic liver disease and progression from steatosis to steatohepatitis[3-6]

- ALN-HSD is an investigational, subcutaneously administered, N-acetylgalactosamine (GalNAc)-conjugated RNA interference therapeutic targeting liver-expressed *HSD17B13* mRNA, in development for the treatment of NASH



References: 1. Singh S et al. *Clin Gastroenterol Hepatol*. 2015. 2. Younossi ZM et al. *Hepatology.* 2016. 3. Ting et al. *Clin Mol Hepatol*. 2021. 4. Luukkonen et al. *CI Insight*. 2020. 5. Pirola et al. *J Lipid Res*. 2019. 6. Abul-Husn et al. *N Engl J Med.* 2018. **Abbreviation:** mRNA, messenger ribonucleic acid.

ALN-HSD Phase 1 Design & Participant Disposition – Part A

Double-blind, placebo-controlled, single ascending dose study in healthy adults

Population (N=58)

- 18–65 years of age
- BMI ≥18 kg/m^2 and ≤28 kg/m^2
- 12-lead ECG within normal limits or with no clinically significant abnormalities with a QTcF <450 msec in males or <470 msec in females

3:1 Randomisation ALN-HSD:Placebo[a]

- ALN-HSD 25 mg OR Placebo SC x1
- ALN-HSD 100 mg OR Placebo SC x1
- ALN-HSD 200 mg OR Placebo SC x1[b]
- ALN-HSD 400 mg OR Placebo SC x1[b]
- ALN-HSD 800 mg OR Placebo SC x1

0 – 3 months

▲ = study drug administration

Primary Endpoint
- Frequency of AEs

Secondary Endpoint
- PK measures
  - AUC
  - C_{max}
  - Fraction excreted in urine of ALN-HSD and potential major metabolite(s)

	Placebo (N=14)	Total ALN-HSD (N=44)
Randomised, n (%)	14 (100.0)	44 (100.0)
Treated, n (%)	14 (100.0)	44 (100.0)
Completed study, n (%)	14 (100.0)	44 (100.0)
Withdrew from study, n	0	0

[a]Cohorts were enrolled sequentially. [b]Includes a dedicated Japanese cohort. Data cutoff date: March 21, 2023. NCT04565717.

Abbreviations: AE, adverse event; AUC, area under the concentration-time curve; BMI, body mass index; C_{max}, maximum plasma concentration; ECG, electrocardiogram; PK, pharmacokinetic; QTcF, Fridericia-corrected QT interval; SAD, single ascending dose; SC, subcutaneous.

Baseline Demographics and Characteristics – Part A

Comparable across placebo and ALN-HSD cohorts

	Placebo (N=14)	ALN-HSD							
		25 mg (N=3)	100 mg (N=7)	200 mg (N=6)	400 mg (N=7)	200 mg (Japanese) (N=7)	400 mg (Japanese) (N=7)	800 mg (N=7)	Total ALN-HSD (N=44)
Age, mean (range), years	27.2 (19–43)	24.0 (23–25)	33.6 (23–38)	30.2 (26–34)	27.4 (22–38)	29.9 (23–39)	32.9 (24–41)	25.1 (20–35)	29.4 (20–41)
Male sex, n (%)	8 (57.1)	2 (66.7)	4 (57.1)	3 (50.0)	4 (57.1)	4 (57.1)	4 (57.1)	4 (57.1)	25 (56.8)
White race, n (%)	8 (57.1)	2 (66.7)	6 (85.7)	6 (100.0)	5 (71.4)	0	0	6 (85.7)	25 (56.8)
BMI, mean (SD), kg/m²	22.1 (1.9)	22.9 (2.3)	22.0 (1.9)	22.3 (1.4)	22.6 (1.8)	21.5 (1.5)	21.4 (1.6)	22.0 (2.0)	22.0 (1.7)
ALT, mean (SD), U/L[a]	18.1 (6.6)	15.3 (4.2)	22.7 (15.4)	17.5 (7.7)	21.0 (11.3)	18.0 (4.7)	14.7 (3.9)	18.3 (5.6)	18.5 (8.7)
AST, mean (SD), U/L[b]	16.1 (2.4)	16.0 (1.7)	20.6 (5.8)	16.7 (4.0)	19.7 (5.4)	17.6 (2.3)	13.1 (1.1)	20.6 (5.7)	17.9 (4.8)

[a]ALT normal range: 10-35 U/L (female), 10-50 U/L (male). [b]AST normal range: 0-31 U/L (female), 0-37 U/L (male). Data cutoff date: March 21, 2023.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; SD, standard deviation.

Safety Summary – Part A

- All TEAEs were mild or moderate in severity
- Most common TEAE was injection-site reaction (11.4% of total ALN-HSD group)
  - All were mild in severity and transient
- 1 serious TEAE of tonsillitis occurred, graded mild and deemed unrelated to ALN-HSD
- No deaths or treatment-related serious TEAEs were observed
- No TEAEs of clinical interest, including elevations in ALT or AST

At least 1 TEAE, n (%)[a]	Placebo (N=14)	Total ALN-HSD (N=44)
TEAEs	3 (21.4)	17 (38.6)
TEAEs occurring in ≥10% of either group and at a higher rate in ALN-HSD than placebo		
Injection-site reaction	0	5 (11.4)
Serious TEAE	0	1 (2.3)
Severe TEAE	0	0
TEAE leading to study withdrawal	0	0
TEAE of clinical interest[b]	0	0
Death[c]	0	0

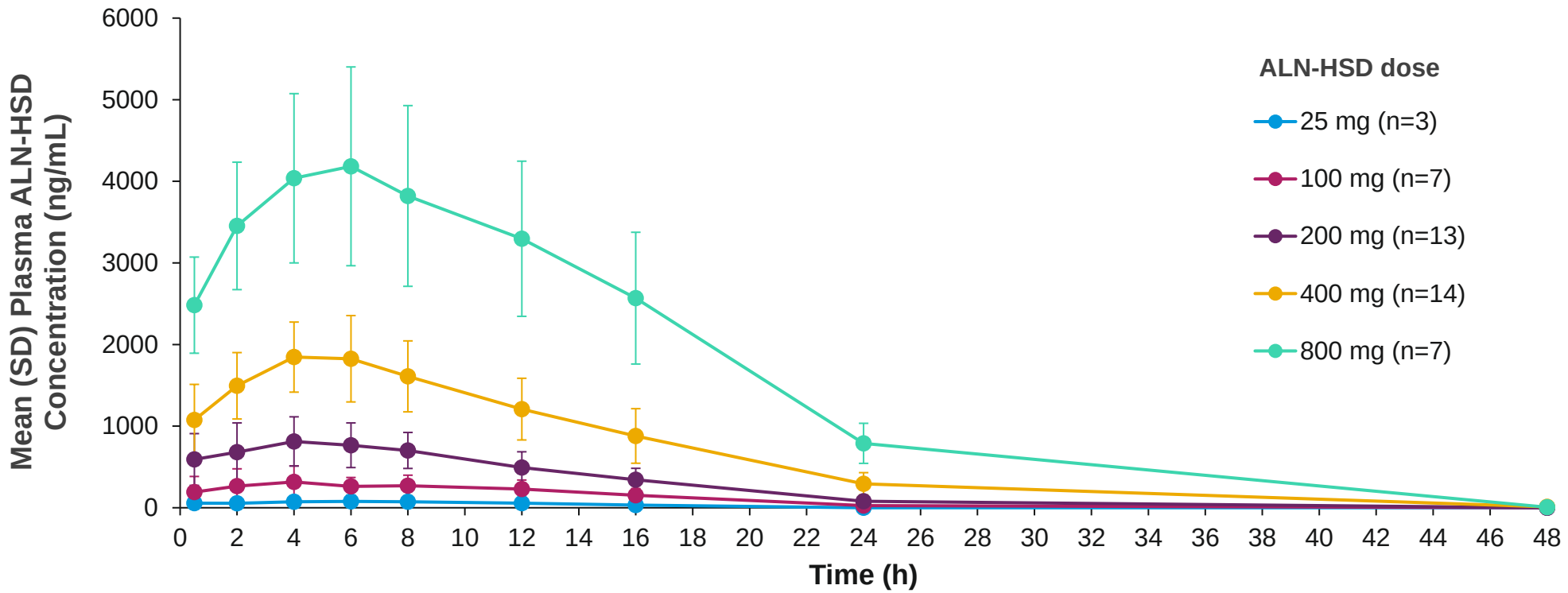
[a]Safety data reported over the 3-month double-blind period. [b]AEs of clinical interest: ALT or AST >3×ULN for participants with normal ALT and AST values at baseline, and >3×baseline in patients with NASH with elevated ALT or AST at baseline, or severe or serious ISRs, ISRs associated with a recall phenomenon (reaction at the site of a prior injection with subsequent injections), or those that lead to temporary dose interruption or permanent discontinuation of ALN-HSD. [c]All deaths included regardless of whether they are treatment-emergent or not. Data cutoff date: March 21, 2023.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ISR, injection site reaction; NASH, nonalcoholic steatohepatitis; TEAE, treatment-emergent adverse event; ULN, upper limit of normal.

ALN-HSD Pharmacokinetics – Part A

- Plasma ALN-HSD levels rapidly declined by 24 hours postdose and were undetectable after 48 hours in most subjects, with terminal half life ranging between 4.2 to 6.7 hours
- ALN-HSD showed a slightly more than dose-proportional increase in exposures across doses
  - Power model estimates for slope (β) (90% CI) were 1.33 (1.23–1.44) and 1.26 (1.15–1.37) for ALN-HSD AUC_{last} and C_{max}, respectively

Mean (SD) Plasma ALN-HSD Concentration (ng/mL) Over Time



- Across doses, ALN-HSD showed 17–37% excretion in urine

Data cutoff date: March 21, 2023.

Abbreviations: AUC_{last}, area under the concentration-time curve from the time of dosing to the last measurable concentration; C_{max}, maximum plasma concentration; SD, standard deviation.

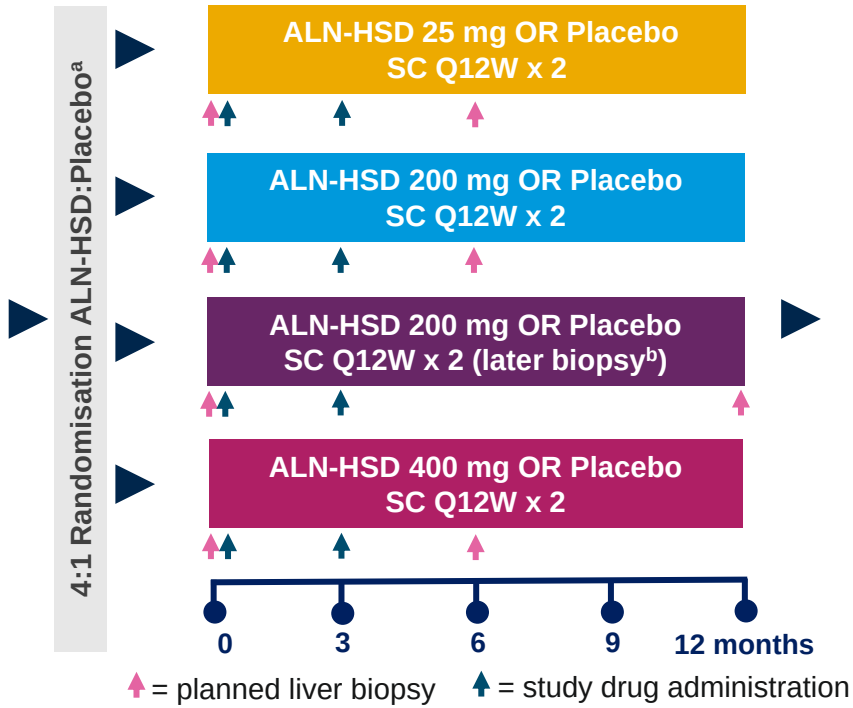
ALN-HSD Phase 1 Design & Patient Disposition – Part B

Double-blind, placebo-controlled, multiple dose study in patients with NASH

- Liver biopsies collected at baseline and post-dose at 6 or 12 months
- Part B of the study was designed to:
  - Test doses predicted to result in 50%, 80%, and 90% maximal knockdown
  - Assess kinetics of recovery from maximal knockdown in a cohort with a later biopsy

Population (N=46)

- 18–65 years of age
- BMI ≥18 kg/m^2 and ≤40 kg/m^2
- Diagnosis of NASH
- Screening liver biopsy that has a NAS score of 3 or more points according to the NASH CRN criteria, including at least 1 point for each of the 3 key NASH histological features, and a fibrosis stage of F0-F3



Primary Endpoint
- Frequency of AEs

Secondary Endpoints
- Plasma concentrations of ALN-HSD and potential major metabolite(s)
- Change from baseline in liver *HSD17B13* mRNA

Exploratory Endpoint
- Change from baseline in NAFLD Activity Score and fibrosis stage

Disposition	Placebo (N=9)	Total ALN-HSD (N=37)
Randomised, n (%)	9 (100.0)	37 (100.0)
Treated, n (%)	9 (100.0)	36 (97.3)
1st dose (Day 1), n (%)	9 (100.0)	36 (97.3)
2nd dose (Day 85), n (%)	9 (100.0)	35 (94.6)
Completed treatment, n (%)	9 (100.0)	35 (94.6)
Discontinued treatment, n (%)	0	1 (2.7)[c]
Completed study, n (%)	8 (88.9)	36 (97.3)
Withdrew from study, n (%)	1 (11.1)[d]	1 (2.7)

[a]Cohorts were enrolled sequentially. Placebo patients were biopsied at 6 or 12 months. [b]Biopsy occurred at 12 months instead of 6 months. [c]1 patient discontinued due to increased liver function tests (due to Hepatitis E). [d]Primary reason for study withdrawal: lost to follow-up. Data cutoff date: March 21, 2023. NCT04565717.

Abbreviations: AE, adverse event; BMI, body mass index; CRN, Clinical Research Network; M, month; mRNA, messenger ribonucleic acid; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; PK, pharmacokinetic; Q12W, every 12 weeks; SC, subcutaneous.

Baseline Demographics and Disease Characteristics – Part B

Comparable across placebo and ALN-HSD cohorts

	Placebo (N=9)	ALN-HSD				
		25 mg (N=8)	200 mg (N=8)	200 mg (later biopsy) (N=12)	400 mg (N=8)	Total ALN-HSD (N=36)
Age, mean (range), years	52.3 (35–64)	56.1 (43–64)	56.8 (42–64)	54.8 (38–64)	55.0 (34–64)	55.6 (34–64)
Male sex, n (%)	4 (44.4)	3 (37.5)	6 (75.0)	6 (50.0)	5 (62.5)	20 (55.6)
White race, n (%)	7 (77.8)	8 (100.0)	4 (50.0)	10 (83.3)	6 (75.0)	28 (77.8)
BMI, mean (SD), kg/m^2	34.9 (4.6)	34.5 (4.0)	33.1 (5.1)	33.6 (3.4)	33.0 (2.5)	33.5 (3.7)
ALT, mean (SD), U/L[a]	57.6 (33.9)	56.8 (26.9)	60.3 (29.1)	44.2 (18.8)	39.9 (17.1)	49.6 (23.5)
AST, mean (SD), U/L[b]	41.0 (21.9)	47.6 (25.4)	41.1 (21.9)	30.6 (8.9)	31.3 (9.7)	36.9 (17.9)
Cholesterol, mean (SD), mmol/L	5.47 (1.23)	5.35 (1.11)	4.78 (0.87)	4.73 (1.17)	4.58 (1.09)	4.84 (1.07)
Triglycerides, mean (SD), mmol/L	2.85 (1.38)	1.81 (0.71)	1.69 (0.80)	1.55 (0.66)	2.47 (1.81)	1.84 (1.07)
Hemoglobin A1C, mean (SD), %	6.5 (0.9)	6.2 (1.2)	7.4 (1.2)	5.9 (0.9)	6.2 (0.9)	6.4 (1.2)
NAFLD activity score, mean (SD)	4.2 (0.8)	4.3 (0.9)	4.0 (0.9)	4.6 (1.1)	4.5 (1.1)	4.4 (1.0)
Fibrosis stage, mean (SD)	1.6 (0.7)	1.9 (0.6)	1.5 (0.9)	1.7 (0.9)	1.6 (0.7)	1.7 (0.8)

- No patients had homozygous protective alleles[c] for any of the 3 variants of *HSD17B13*
- Similar proportions of patients in the placebo and ALN-HSD groups were heterozygous or homozygous for the *PNPLA3* I148M risk allele

[a]ALT normal range: ≤33 U/L (female), ≤41 U/L (male). [b]AST normal range: ≤31 U/L (female), ≤37 U/L (male). [c]Alleles used: rs62305723:A, rs72613567:TA, and rs80182459:G. Data cutoff date: March 21, 2023.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; NAFLD, nonalcoholic fatty liver disease; SD, standard deviation.

Safety Summary – Part B

- Majority of TEAEs were mild or moderate in severity
- The only TEAE occurring in ≥10% of patients was COVID-19 (13.9% of total ALN-HSD group), all events deemed unrelated to study drug
- No deaths or treatment-related serious TEAEs were observed
- Two severe TEAEs were deemed unrelated to study drug
- No interruptions or discontinuations related to study drug were observed
- No cases of Hy's law were observed
- There was no evidence of drug-induced liver injury

At least 1 TEAE, n (%)	Placebo (N=9)	Total ALN-HSD (N=36)
TEAEs	7 (77.8)	29 (80.6)
TEAEs occurring in ≥10% of either group and at a higher rate in ALN-HSD than placebo		
COVID-19	0	5 (13.9)
Serious TEAE	0	1 (2.8)[a]
Severe TEAE	0	2 (5.6)[b]
TEAE leading to study drug discontinuation	0	1 (2.8)[c]
TEAE leading to study withdrawal	0	0
TEAE of clinical interest[d]	0	1 (2.8)[e]
Death[f]	0	0

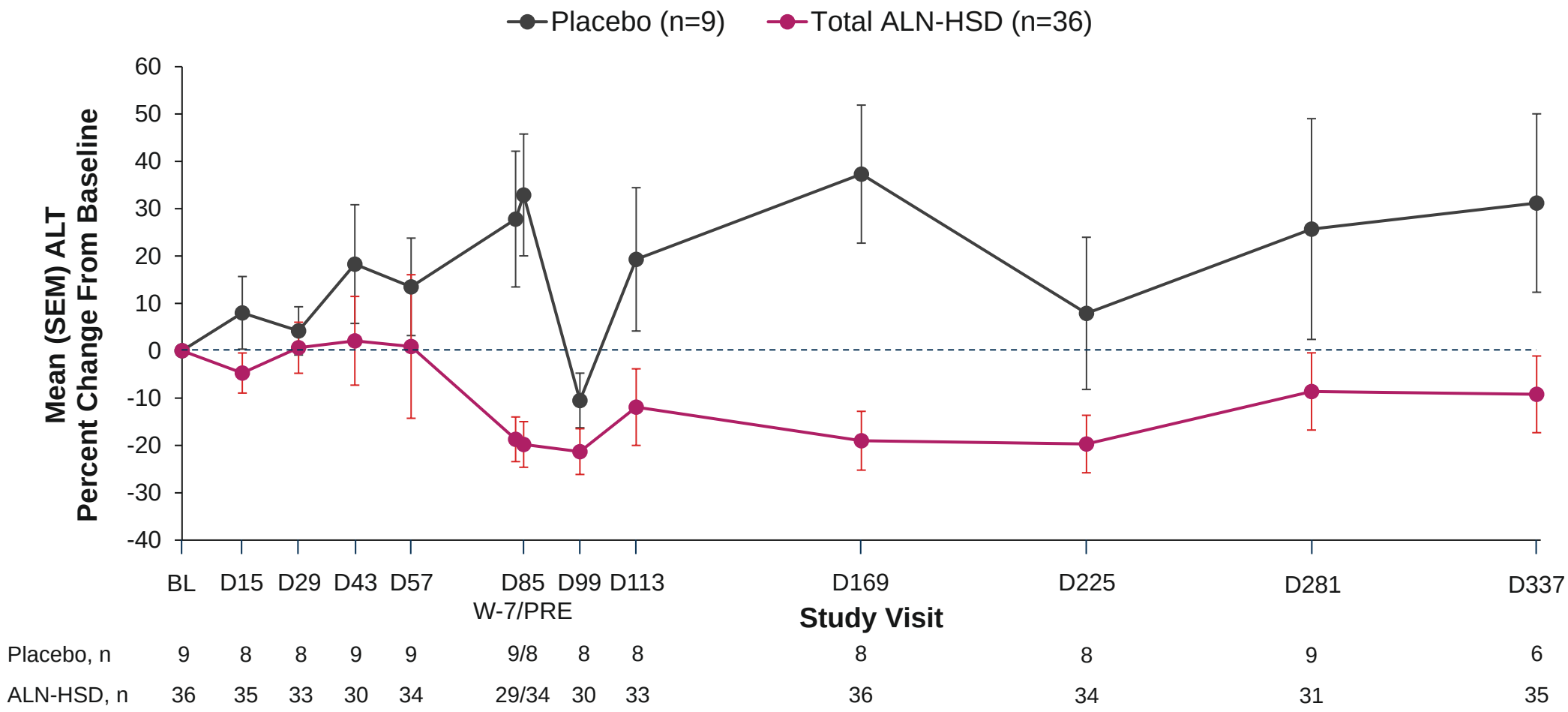
[a]1 patient had appendicitis. [b]1 patient had appendicitis, and 1 patient had a tooth infection. [c]1 patient had increased liver function tests (due to Hepatitis E). [d]AEs of clinical interest: ALT or AST >3×ULN for participants with normal ALT and AST values at baseline, and >3×baseline in patients with NASH 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 lead to temporary dose interruption or permanent discontinuation of ALN-HSD. [e]1 patient had increased liver function test (due to Hepatitis E); none of the ISR cases met the criteria for clinical interest. [f]All deaths were included regardless of whether they were treatment-emergent or not. Data cutoff date: March 21, 2023.

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ISR, injection site reaction; NASH, nonalcoholic steatohepatitis; TEAE, treatment-emergent adverse event; ULN, upper limit of normal.

Trend Towards Improvement in Liver Enzymes – Part B

ALN-HSD was associated with numerically lower ALT levels over time compared with placebo

Mean (SEM) Percent Change from Baseline in ALT Levels



	BL	D15	D29	D43	D57	D85 W-7/PRE	D99	D113	D169	D225	D281	D337
Placebo, n	9	8	8	9	9	9/8	8	8	8	8	9	6
ALN-HSD, n	36	35	33	30	34	29/34	30	33	36	34	31	35

Mean (SD) values at baseline were 57.6 (33.9) U/L for placebo and 49.6 (23.5) U/L for total ALN-HSD. Data cutoff date: March 21, 2023.

Abbreviations: ALT, alanine aminotransferase; BL, baseline; D, day; SEM, standard error of the mean; W-7/PRE, within 7 days/pre-dose.

HSD mRNA Reduction in Liver Biopsies at M6 or M12 – Part B

ALN-HSD was associated with dose-dependent reduction of *HSD17B13* mRNA

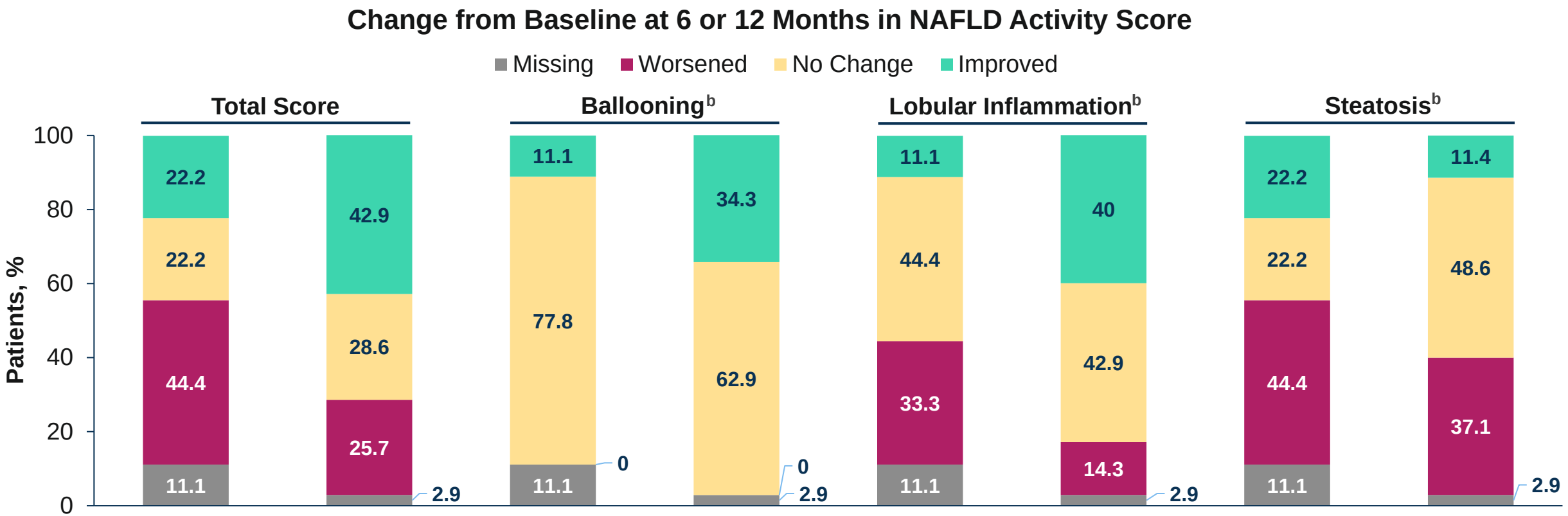
	Placebo (N=9)	ALN-HSD		
		25 mg (N=8)	200 mg (N=20)	400 mg (N=7)
Month 6 mRNA Percent Change from Baseline				
N	5	8	9	7[a]
Mean	4.6	-38.7	-68.5	-73.2
Median	4.7	-39.8	-71.4	-78.3
Range	-11.7, 18.1	-67.2, -4.5	-79.3, -55.8	-80.9, -53.7
Month 12 mRNA Percent Change from Baseline				
n	3	0[b]	10	0[b]
Mean	-0.4	N/A	-34.3	N/A
Median	-1.1	N/A	-30.6	N/A
Range	-3.0, 3.0	N/A	-64.4, 4.0	N/A

[a]Percent change from baseline of -53.7% was observed in the patient who received only the first dose of ALN-HSD. [b]Not evaluated at 12 months. No patients had homozygous protective alleles for any of the 3 variants of *HSD17B13.* Data cutoff date: March 21, 2023.

Abbreviations: M, month; mRNA, messenger ribonucleic acid; N/A, not applicable; SD, standard deviation.

Categorical Change from Baseline in Histologic Parameters – Part B

ALN-HSD was associated with numerically lower biopsy-derived NAFLD activity score over 6 or 12 months[a] relative to placebo

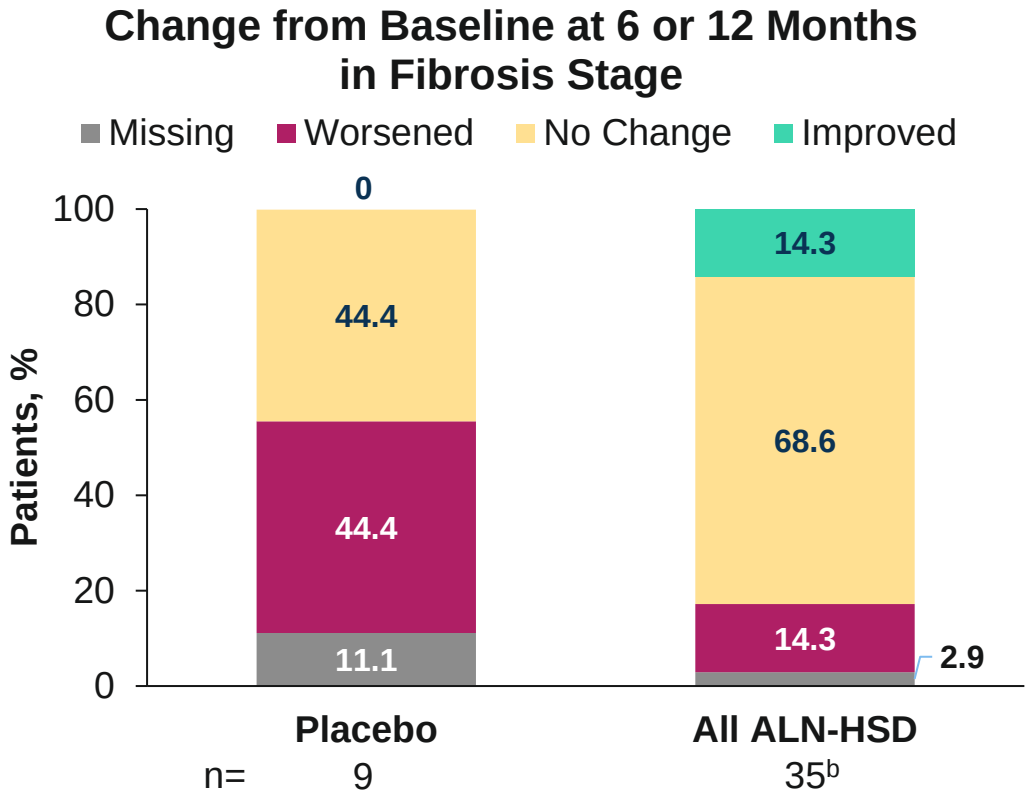


[a]12-month biopsies were only available in one of the two 200 mg cohorts. [b]Subcomponent of NAFLD total activity score. [c]Second biopsy visit was cancelled in one patient out of 36 in the ALN-HSD group. Data cutoff date: March 21, 2023. Worsened: change from baseline >0. Improved: change from baseline <0.

Abbreviation: NAFLD, nonalcoholic fatty liver disease; SD, standard deviation.

Categorical Change from Baseline in Histologic Parameters – Part B

ALN-HSD was associated with numerically lower fibrosis stage over 6 or 12 months[a] relative to placebo



[a]12-month biopsies were only available in one of the two 200 mg cohorts. [b]Second biopsy visit was cancelled in one patient out of 36 in the ALN-HSD group. Data cutoff date: March 21, 2023. Worsened: change from baseline >0. Improved: change from baseline <0. **Abbreviation:** SD, standard deviation.

Summary

- In this phase 1 study, ALN-HSD exhibited an encouraging safety and tolerability profile in healthy adults and in patients with NASH
  - The majority of AEs were mild or moderate, and no treatment-related serious or severe AEs were reported during the duration of the study
- In Part A, ALN-HSD demonstrated a slightly more than dose-proportional increase in exposure
  - Across doses, ALN-HSD showed 17–37% excretion in urine
- In Part B, ALN-HSD showed robust dose-dependent reductions in *HSD17B13* mRNA expression in liver biopsies
  - ALN-HSD was also associated with numerically lower liver enzymes, biopsy-derived NAFLD activity score, and fibrosis stage over 6 or 12 months relative to placebo
- A phase 2 study has been initiated to further investigate ALN-HSD as a potential therapy in patients with NASH (NCT05519475)

Abbreviations: AE, adverse event; mRNA, messenger ribonucleic acid; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

Thank you to the patients, their families, investigators, study staff, and collaborators for their participation in the ALN-HSD-001 study