Supplementary appendix

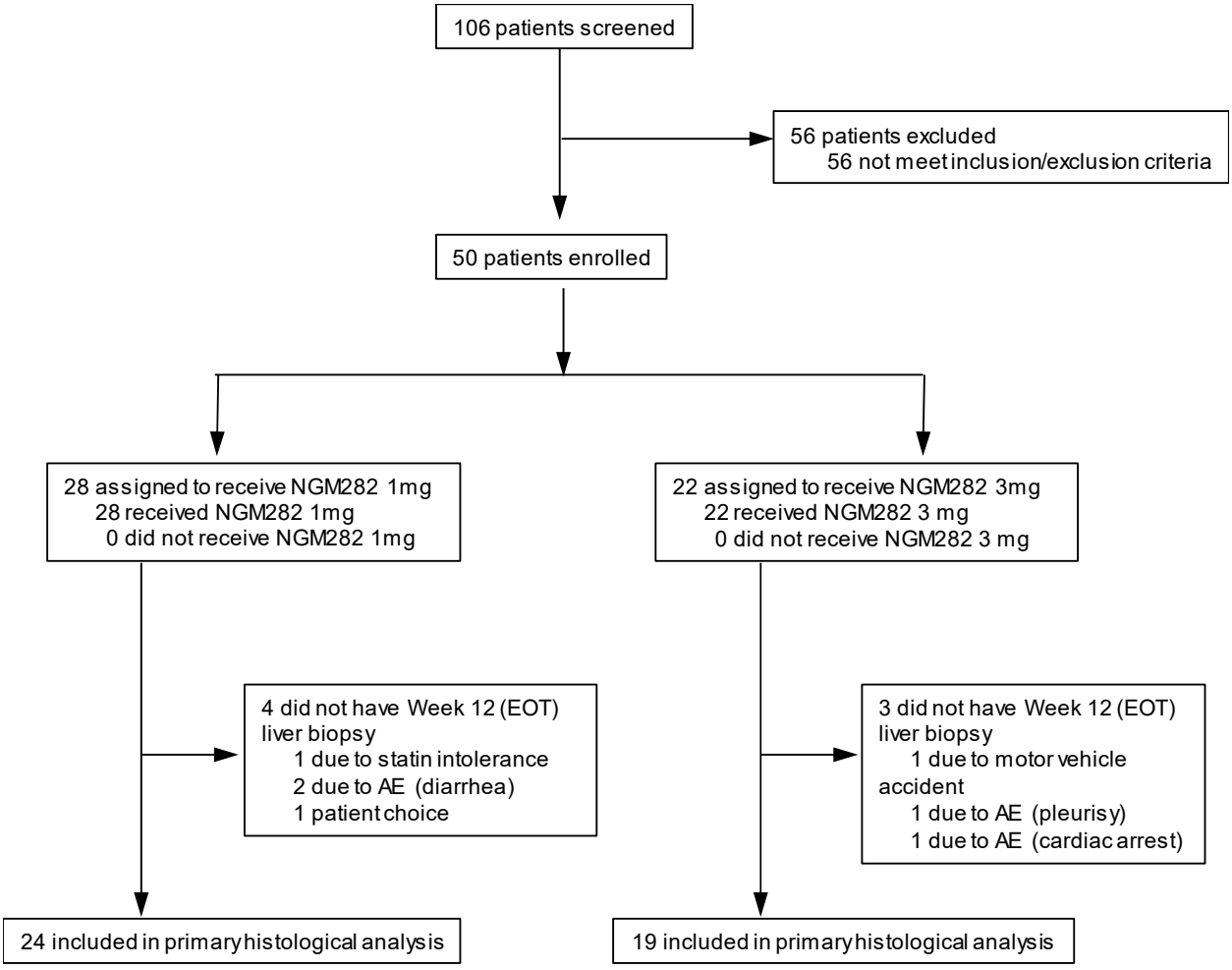
Supplement to: Stephen A. Harrison et al., NGM282 Improves Liver Fibrosis and Histology in 12 Weeks in Patients with Nonalcoholic Steatohepatitis

Corresponding Author:

Stephen A. Harrison, MD; Radcliffe Department of Medicine, University of Oxford, United Kingdom; and Pinnacle Clinical Research, 5109 Medical Drive, Suite 316, San Antonio, TX 78229; Phone: (210) 982-0320 Ext 1457; Email: stephenharrison87@gmail.com

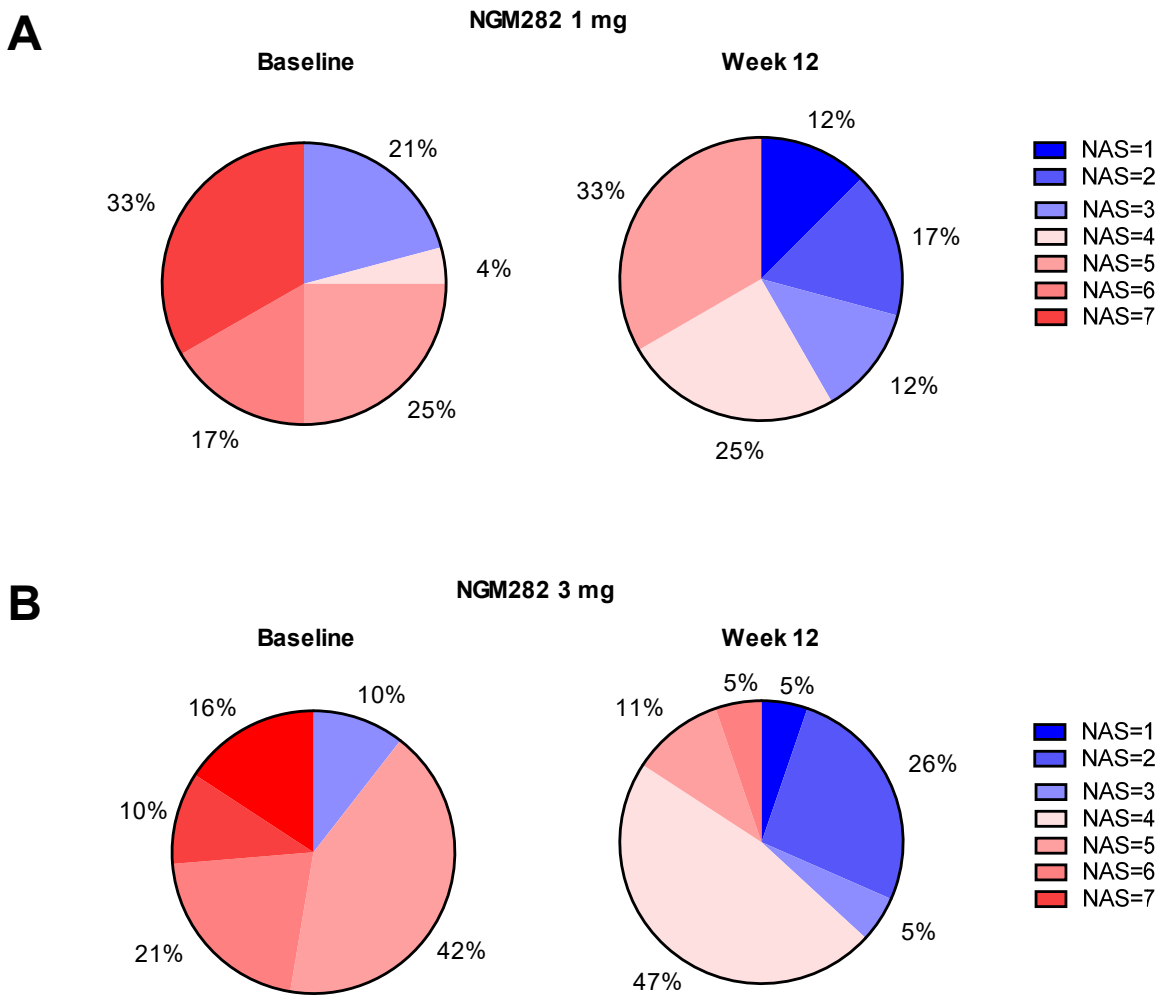
SUPPLEMENTARY APPENDIX

Supplemental Figures



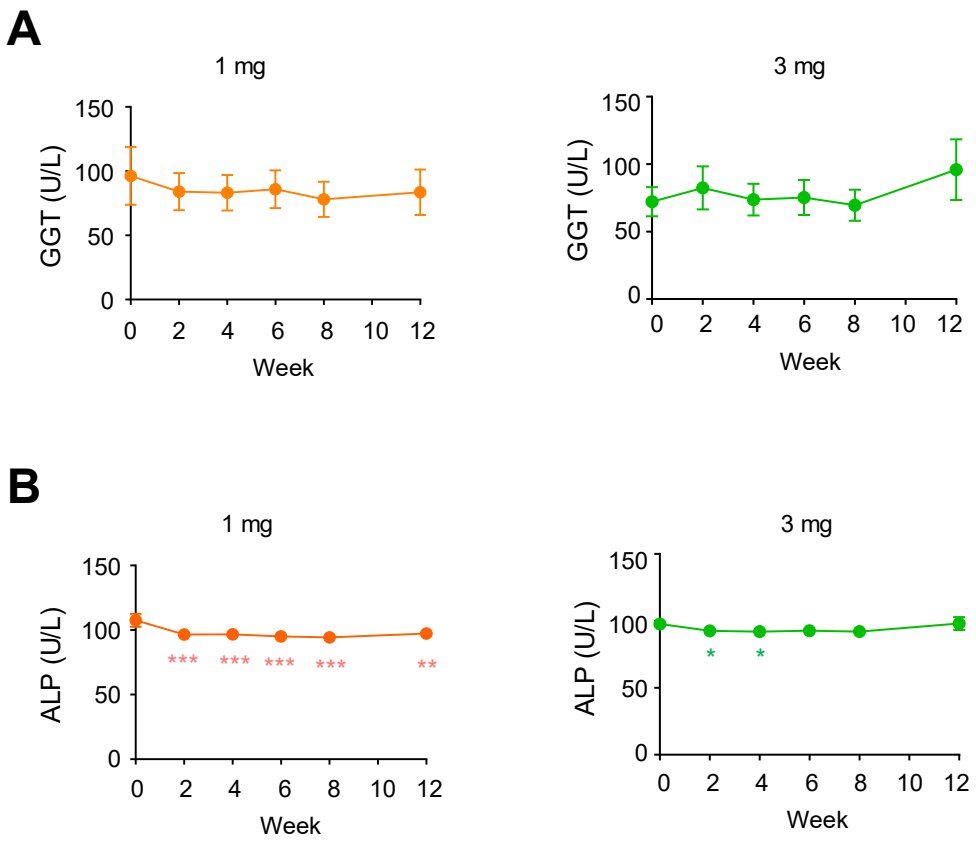
Supplementary Fig. S1. Trial profile.

AE, adverse event; EOT, end of treatment



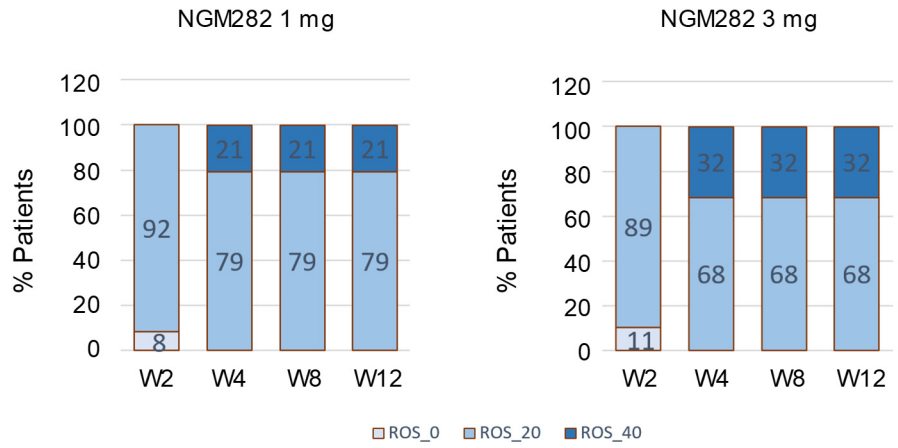
Supplementary Fig. S2. Distributions of total NAS scores at baseline and week 12.

(A) Distributions of total NAS scores at baseline and week 12 in the NGM282 1 mg group. (B) Distributions of total NAS scores at baseline and week 12 in the NGM282 3 mg group. Proportions of patients with total NAS scores of 1-7 are presented. No patients had a NAS score of 0 or 8 at baseline or week 12. NAS, nonalcoholic fatty liver disease activity score.



Supplementary Fig. S3. GGT and ALP over time

(A) Serum concentrations of GGT over time. **(B)** Serum concentrations of ALP over time. Panels show the concentrations of GGT and ALP measured at baseline, weeks 2, 4, 6, 8 and 12 in patients treated with NGM282 1 mg or 3 mg. ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase. ***P<0.001, **P<0.01, *P<0.05 vs baseline by one-sample t-test.



Supplementary Fig. S4. Rosuvastatin co-administration in the NGM282 1 mg and 3 mg groups

Patients received NGM282 at a dose of 1 mg or 3 mg per day subcutaneously for 2 weeks, before the biweekly dose escalation of rosuvastatin to a target dose of 40 mg per day began. Patients underwent lipid management according to the algorithm (Rinella et al., J Hepatology 2018). A value of 100% represents all participants who had paired liver biopsies in each dose group. Shown are proportions of patients with rosuvastatin co-administration at weeks 2, 4, 8 and 12. Percentages may not total 100 because of rounding. ROS_0, no rosuvastatin; ROS_20, rosuvastatin 20 mg; ROS_40, rosuvastatin 40 mg.

Supplemental Tables

Supplementary Table S1. List of patients who were not included in the modified intention-to-treat (mITT) analysis

Patient ID	First Biopsy at Baseline	Second Biopsy at Week 12	Reason
NGM282 1 mg			
1 mg WD#1	Yes	No	Patients declined second biopsy due to statin intolerance
1 mg WD#2	Yes	No	Patient withdrew consent, no longer wanted to participate in the trial
1 mg WD#3	Yes	No	Patient discontinued study drug on Day 33 due to adverse event (diarrhea)
1 mg WD#4	Yes	No	Patient discontinued study drug on Day 29 due to adverse event (diarrhea)
NGM282 3 mg			
3 mg WD#1	Yes	No	Patient discontinued study drug on Day 50 due to a motor vehicle accident
3 mg WD#2	Yes	No	Patient discontinued study drug on Day 28 due to a serious adverse event (pleurisy)
3 mg WD#3	Yes	No	Patient discontinued study drug on Day 28 due to a serious adverse event (cardiac arrest)

Supplementary Table S2. Sensitivity analysis of changes from baseline to week 12 in key outcomes using Wilcoxon matched-pairs test

	NGM282 1 mg (n=24)		NGM282 3 mg (n=19)	
	Change from Baseline to Week 12	**p**	**Change from Baseline to Week 12**	**p**
Histology				
Total NAS score	−2 (−2.6, −1.2)	<0.001	−2 (−3.1, −1.3)	<0.001
Baseline NAS 3-6	−1 (−2.1, −0.4)	0.008	−1.5 (−2.3, −0.7)	0.003
Baseline NAS 7-8	−3 (−4.1, −2.2)	0.008	−4 (−6.0, −2.4)	0.06
Steatosis	−1 (−1.4, −0.7)	<0.001	−1 (−1.5, −0.7)	<0.001
Ballooning	0 (−0.8, 0)	0.044	−1 (−1.1, −0.2)	0.008
Inflammation	0 (−0.6, −0.1)	0.008	0 (−0.8, −0.1)	0.031
Total Fibrosis score	0 (−0.4, 0.2)	0.59	0 (−0.9, −0.04)	0.057
Baseline F1	0.5 (−0.4, 1.4)		0 (−1.1, 1.8)	
Baseline F2	0 (−0.8, 0.4)		0 (−1.1, 0.3)	
Baseline F3	0 (−0.6, 0.1)		−0.5 (−1.4, 0)	
Baseline F4	−1		−1	
Imaging				
Liver fat content by MRI-PDFF (%)	−10.2 (−13.0, −8.8)	<0.001	−10.4 (−13.3, −9.2)	<0.001
cT1 by Liver*MultiScan*	−76 (−102.6, −53.2)	<0.001	−72 (−110.1, −53.1)	<0.001
Serum markers of target engagement				
C4 (ng/mL)	−19.6 (−43.9, −17.9)	<0.001	−28.1 (−45.0, −21.4)	<0.001
Total bile acids (µmol/L)	−2.4 (−5.7, −1.5)	<0.001	−3.6 (−5.5, −2.4)	<0.001
Liver enzymes				
ALT (U/L)	−49 (−81.5, −46.1)	<0.001	−47 (−70.2, −35.6)	<0.001
AST (U/L)	−38 (−56.7, −28.2)	<0.001	−35 (−50.0, −24.8)	<0.001
Serum fibrosis markers				
Pro-C3 (ng/mL)	−2.6 (−7.5, −1.5)	<0.001	−6.5 (−19.9, −2.2)	0.001
ELF score	−0.3 (−0.5, −0.2)	<0.001	−0.7 (−0.8, −0.3)	<0.001
Hyaluronic acid (UG/L)	−2.5 (−11.0, 12.4)	0.77	−5 (−43.6, 5.1)	0.12
PIIINP (UG/L)	−2.4 (−4.6, −1.5)	<0.001	−3.7 (−4.7, −1.3)	0.002
TIMP-1 (UG/L)	−40 (−51.6, −27.7)	<0.001	−37 (−58.9, −18.7)	<0.001

Shown are median (95% CI). P values by Wilcoxon matched-pairs signed rank test.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; ELF, enhanced liver fibrosis; MRI-PDFF, magnetic resonance imaging- proton density fat fraction; NAS, nonalcoholic fatty liver disease activity score; PIIINP, N-terminal pro-peptide of collagen III; Pro-C3, neoepitope-specific N-terminal pro-peptide of type III collagen; SD, standard deviation; TIMP-1, tissue inhibitor of metalloproteinase 1

Supplementary Table S3. Sensitivity analysis of changes from baseline to week 12 in histological outcomes in the NGM282 1 mg group excluding 6 patients

	NGM282 1 mg (n=18)			
	Baseline	**Week 12**	**Change from Baseline to Week 12**	**p**
Histology				
Total NAS score	6.0 (1.0)	3.7 (1.4)	−2.3 (1.6)	<0.001
Baseline NAS 4-6	5.3 (0.6)	3.6 (1.6)	−1.6 (1.5)	0.005
Baseline NAS 7-8	7.0 (0)	3.9 (1.1)	−3.1 (1.1)	<0.001
Steatosis	2.2 (0.9)	0.9 (0.5)	−1.3 (0.8)	<0.001
Ballooning	1.7 (0.5)	1.1 (0.9)	−0.6 (0.8)	0.007
Inflammation	2.0 (0.4)	1.7 (0.5)	−0.3 (0.5)	0.010
Total Fibrosis score	2.3 (0.7)	2.2 (0.9)	−0.1 (0.8)	0.54
Baseline F1	1.0 (0)	1.5 (0.7)	0.5 (0.7)	
Baseline F2	2.0 (0)	1.9 (1.0)	−0.1 (1.0)	
Baseline F3	3.0 (0)	2.8 (0.5)	−0.2 (0.5)	
Histological Response		*10 (56%)*		
NAS≥2 reduction without fibrosis worsening		*11 (61%)*		
Fibrosis ≥1 reduction without NASH worsening		4 (22%)		
NASH resolution without fibrosis worsening		*3 (17%)*		

6 patients in the NGM282 1 mg cohort who no longer met inclusion criteria (NAS≥4, with ≥1 in each component; F1-F3) upon central pathology review were excluded for this sensitivity analysis. Among the 6 patients, 1 had F4, 5 had NAS=3 (4 of the 5 had no ballooning) in the baseline biopsies upon central read.

Shown are mean (SD) or n (%). P values by one-sample t-test. Histological response is defined as 2-point or greater improvement in NAS without worsening of fibrosis, or improvement in fibrosis of 1-stage or more without worsening of NASH (defined as no increase in NAS for ballooning, inflammation or steatosis). Resolution of NASH is defined as a score of 0–1 for inflammation, 0 for ballooning, and any value for steatosis.

NAS, nonalcoholic fatty liver disease activity score; SD, standard deviation

Supplementary Table S4. Sensitivity analysis of changes from baseline to week 12 in histological outcomes in the NGM282 3 mg group excluding 3 patients

	NGM282 3 mg (n=16)			
	Baseline	**Week 12**	**Change from Baseline to Week 12**	**p**
Histology				
Total NAS score	6.1 (1.2)	3.4 (1.3)	−2.6 (1.6)	<0.001
Baseline NAS 4-6	5.4 (0.5)	3.4 (1.4)	−1.9 (1.1)	<0.001
Baseline NAS 7-8	7.6 (0.5)	3.4 (1.3)	−4.2 (1.5)	0.003
Steatosis	1.8 (0.8)	0.5 (0.5)	−1.2 (0.8)	<0.001
Ballooning	1.9 (0.2)	1.1 (0.8)	−0.9 (0.8)	<0.001
Inflammation	2.4 (0.6)	1.8 (0.5)	−0.6 (0.8)	0.014
Total Fibrosis score	2.6 (0.6)	2.0 (1.0)	−0.6 (0.9)	0.024
Baseline F1	1.0 (0)	1.0 (0)	0 (0)	
Baseline F2	2.0 (0)	1.6 (0.5)	−0.4 (0.5)	
Baseline F3	3.0 (0)	2.3 (1.0)	−0.7 (1.0)	
Histological Response		*12 (75%)*		
NAS≥2 reduction without fibrosis worsening		*12 (75%)*		
Fibrosis ≥1 reduction without NASH worsening		*7 (44%)*		
NASH resolution without fibrosis worsening		*2 (12%)*		

3 patients in the NGM282 3 mg cohort who no longer met inclusion criteria (NAS≥4, with ≥1 in each component; F1-F3) upon central pathology review were excluded for this sensitivity analysis. Among the 3 patients, 1 had F4, 2 had NAS=3 (1 of the 2 had no ballooning) in the baseline biopsies upon central read.

Shown are mean (SD) or n (%). P values by one-sample t-test. Histological response is defined as 2-point or greater improvement in NAS without worsening of fibrosis, or improvement in fibrosis of 1-stage or more without worsening of NASH (defined as no increase in NAS for ballooning, inflammation or steatosis). Resolution of NASH is defined as a score of 0–1 for inflammation, 0 for ballooning, and any value for steatosis.

NAS, nonalcoholic fatty liver disease activity score; SD, standard deviation

Supplementary Table S5. Between group differences of change from baseline to week 12 in C4 and liver fat content

	LS mean Differences (95% CI) in Change from Baseline to Week 12	
	NGM282 1 mg vs 3 mg	**P**
C4 (ng/mL)	5.0 (0.2 to 9.7)	0.041
LFC by MRI-PDFF (%)	1.2 (−1.1 to 3.4)	0.30

To compare across treatment groups in changes from baseline to week 12 (end of treatment), we used analysis of covariance (ANCOVA) with treatment group and baseline value as covariates at the 5% level of significance. CI, confidence interval; LFC, liver fat content; LS, least squares; MRI-PDFF, magnetic resonance imaging- proton density fat fraction