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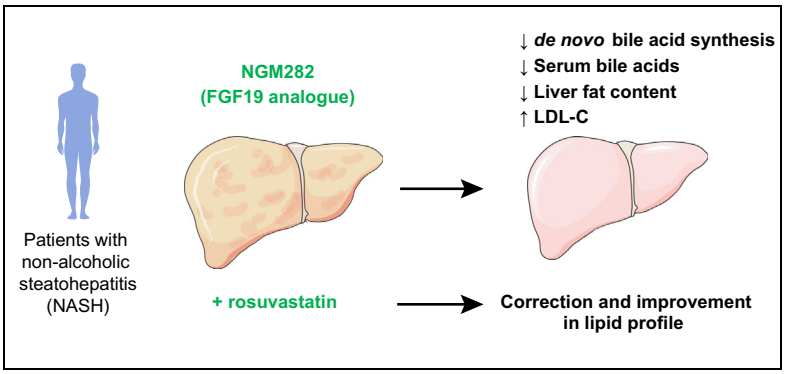
Graphical abstract



Highlights

- NGM282 is a first-in-class, engineered analogue of the gut hormone FGF19.
- NGM282 therapy is associated with an elevation of blood cholesterol in patients with NASH.
- Co-administration of rosuvastatin significantly reduces LDL-C, LDL particles, triglycerides and VLDL particles.
- Co-administration of rosuvastatin results in increases in HDL-C and HDL particles.

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Lay summary

Non-alcoholic steatohepatitis (NASH) represents a large and growing public health concern with no approved therapy. NGM282, an engineered analogue of the gut hormone FGF19, reduces liver fat, liver injury and inflammation in patients with NASH. However, NGM282 increases cholesterol levels. Here we show that co-administration of a statin can manage the cholesterol increase seen in patients with NASH receiving treatment with NGM282, producing a favorable overall lipid profile.

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Rosuvastatin improves the FGF19 analogue NGM282-associated lipid changes in patients with non-alcoholic steatohepatitis

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Background: NGM282, an engineered analogue of the gut hormone FGF19, improves hepatic steatosis and fibrosis biomarkers in patients with non-alcoholic steatohepatitis (NASH). However, NGM282 increases serum cholesterol levels by inhibiting CYP7A1, which encodes the rate-limiting enzyme in the conversion of cholesterol to bile acids. Herein, we investigate whether administration of a statin can manage the cholesterol increase seen in patients with NASH receiving treatment with NGM282.

Methods: In this phase II, open-label, multicenter study, patients with biopsy-confirmed NASH were treated with subcutaneous NGM282 once daily for 12 weeks. After 2 weeks, rosuvastatin was added in stepwise, biweekly incremental doses to a maximum of 40 mg daily. Both drugs were continued until the end of treatment at week 12. We evaluated plasma lipids, lipoprotein particles and liver fat content.

Results: In 66 patients who received NGM282 0.3 mg (n = 23), NGM282 1 mg (n = 21), or NGM282 3 mg (n = 22), circulating cholesterol increased from baseline at week 2. Initiation of rosuvastatin resulted in rapid decline in plasma levels of total cholesterol and low-density lipoprotein cholesterol. At week 12, reductions from baseline in total cholesterol levels of up to 18% (p <0.001), low-density lipoprotein cholesterol of up to 28% (p <0.001), triglycerides of up to 34% (p <0.001) and an increase in high-density lipoprotein cholesterol of up to 16% (p <0.001), with similar changes in lipoprotein particles, were observed in these patients. Robust decreases from baseline in 7alpha-hydroxy-4-cholesten-3-one (p <0.001) and liver fat content (p <0.001) were also observed. Rosuvastatin was safe and well-tolerated when co-administered with NGM282 in patients with NASH.

Conclusions: In this multicenter study, NGM282-associated elevation of cholesterol was effectively managed with rosuvastatin. Co-administration of rosuvastatin with NGM282 may be a reasonable strategy to optimize the cardiovascular risk profile in patients with NASH.

Lay summary: Non-alcoholic steatohepatitis (NASH) represents a large and growing public health concern with no approved therapy. NGM282, an engineered analogue of the gut hormone FGF19, reduces liver fat, liver injury and inflammation in patients with NASH. However, NGM282 increases cholesterol levels. Here we show that co-administration of a statin can manage the cholesterol increase seen in patients with NASH receiving treatment with NGM282, producing a favorable overall lipid profile.

Introduction

Non-alcoholic steatohepatitis (NASH), a severe form of non-alcoholic fatty liver disease (NAFLD), represents a large and growing public health concern that is increasingly contributing to the rising prevalence of cirrhosis and hepatocellular carcinoma globally.[1,2] Currently, there is no approved drug for NASH, which is projected to be the leading indication for liver transplantation in the next decade.[3] The pathogenesis of NASH is complex, and it is hypothesized that toxic lipid species or intermediates may inflict hepatocyte injury.[4] In recent years, bile acids have emerged as important molecules that act at both hepatic and extrahepatic tissues to modulate metabolic, inflammation and fibrogenesis pathways.[5] While crucial for the emulsification and absorption of dietary fat, bile acids can cause cell death and liver injury when amassed within hepatocytes. Indeed, patients with NASH have elevated hepatic and circulating concentrations of bile acids.[6,7]

Fibroblast growth factor 19 (FGF19), an endocrine hormone produced in the ileum, acts on the liver to suppress bile acid synthesis from cholesterol, while also inhibiting insulin-induced hepatic lipogenesis.[8,9] FGF19 regulates bile acid metabolism via suppression of CYP7A1, the first and rate-limiting enzyme in the classic pathway for the conversion of cholesterol to bile acids. NGM282 is a non-tumorigenic analogue of FGF19 that retains the ability to suppress CYP7A1.[10,11] In a double-blind, randomized, placebo-controlled study previously reported, NGM282 3 mg and 6 mg produced rapid and sustained improvements in liver fat content over 12 weeks in patients with biopsy-proven NASH.[12] NGM282 also significantly improved liver

Keywords: FGF19; Rosuvastatin; Non-alcoholic steatohepatitis; Cholesterol; Triglyceride.
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aminotransferases, serum fibrosis biomarkers, and histological features of NASH.[12,13] However, a significant increase in low-density lipoprotein cholesterol (LDL-C) was observed after 12 weeks of treatment with NGM282. Given that patients with NASH develop atherogenic dyslipidemia, which is associated with an increased risk of atherosclerotic cardiovascular disease (ASCVD),[14–16] it is important to explore whether co-administration of a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor can mitigate the effect of NGM282 on LDL-C and favorably impact the atherogenic lipoprotein profile.

We conducted a phase II, multicenter, open-label trial to better define LDL changes associated with NGM282 and elucidate whether these NGM282-associated increases in total cholesterol and LDL-C could be managed with concomitant statin use in adult patients with NASH.

Patients and methods

Study design and participants

The study protocol and relevant supporting data were approved by the local ethics committees prior to study initiation. The study was conducted in compliance with International Conference on Harmonization, E6 Good Clinical Practice, and all patients provided written informed consent. Dose selection and the 12-week treatment duration were selected based on previous trials investigating the effect of NGM282 on liver fat content.[12]

Detailed patient inclusion and exclusion criteria are provided (Table S1). Patients were eligible if they had NAFLD Activity Score (NAS) ≥4, stage 1, 2, or 3 fibrosis, liver fat content ≥8% as assessed by magnetic resonance imaging-proton density fat fraction (MRI-PDFF), glycosylated hemoglobin ≤9.5%, and LDL-C ≤165 mg/dl. Major exclusion criteria were acute or chronic liver disease unrelated to NASH, evident cardiovascular diseases (myocardial infarction, stroke, unstable angina, arrhythmia, or other cardiac events that required hospitalization or significant clinical intervention) within 6 months of screening, and type 1 diabetes. Individuals on low-dose statin (≤50% of clinically approved maximal dose) could be included but those taking other lipid-lowering drugs including ezetimibe, colesevelam, cholestyramine, niacin or PCSK9 inhibitors were excluded. Additionally, initiating any of these medications during the study period was prohibited. Patients were sequentially enrolled into the study and categorized as either statin-naïve (defined as no administration of statins within 3 months prior to enrollment) or ongoing statin therapy (defined as administration of ≤50% of the maximal dose of currently approved statin therapy) with no more than 8 individuals on ongoing statin therapy within each dosing group. Patients were blinded to dose assignment throughout the study period.

Procedures

During the screening period, patients underwent a liver biopsy or provided a liver biopsy tissue specimen obtained within the previous 12 months. Liver biopsies were read by qualified local pathologists at enrollment, and were centrally read at the completion of the study. Patients received daily subcutaneous NGM282 during weeks 1–2, before being initiated on concurrent treatment with daily rosuvastatin tablets according to the study algorithm (Fig. 1). Patients were to start with 20 mg rosuvastatin if statin naive or switch to 20 mg rosuvastatin if already on statin therapy (acceptable daily doses of approved statin therapies at baseline included: atorvastatin <40 mg, fluvastatin <40 mg, lovastatin <30 mg or <40 mg [immediate release], pitavastatin <2 mg, pravastatin <40 mg, rosuvastatin <20 mg, simvastatin <40 mg), to treat LDL-C elevations of greater than 10 mg/dl from baseline at week 2. Patients could up-titrate to 40 mg rosuvastatin at week 4 and week 8 if LDL-C elevation remained more than 10 mg/dl from baseline. No dose modification on rosuvastatin occurred after week 8.

Patients were evaluated at weeks 1, 2, 4, 6 and 8 for on-treatment assessments, at the end of treatment on week 12, and at follow-up 6 weeks after the last dose. Fasting lipid panel (total cholesterol, LDL-C, high-density lipoprotein cholesterol [HDL-C] and triglycerides) was obtained on day 1, weeks 2, 6, 8, 12 (end of treatment) and 18 (follow-up). Serum levels of 7alpha-hydroxy-4-cholesten-3-one (C4, a marker of bile acid synthesis) and bile acids were measured on day 1, week 6 and week 12 by mass spectrometry methods (Mayo Clinic). Concentrations and size of lipoprotein particles were measured on day 1, weeks 2, 6 and 12 using nuclear magnetic resonance (NMR) method on a Vantera Clinical Analyzer (LabCorp). Liver fat content was measured on day 1 and week 12 by MRI-PDFF as previously described.[12]

Outcomes

The lipid outcome measures were changes in fasting lipids (total cholesterol, LDL-C, HDL-C and triglycerides) and lipoprotein particles from baseline to week 12 (end of treatment). Pharmacodynamic outcome measures were changes in C4 and bile acids from baseline to week 12. The imaging outcome measure was a change from baseline to week 12 in liver fat content as determined by MRI-PDFF.

Statistical analysis

The sample size for this open-label study was based on the results of the double-blind, placebo-controlled cohort previously reported.[12] The efficacy population was used to assess continuous endpoints and included all enrolled patients who received at least one dose (full or partial) of study drug and had both baseline and week 12 lipid values.

Within each treatment group, the change in continuous outcomes from baseline over time was analyzed using one-sample *t* test. 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. When indicated, the change in continuous outcomes *vs.* week 2 (to evaluate the effect of rosuvastatin) was analyzed using one-sample *t* test within each treatment group. Means with standard deviations (SD) and the corresponding *p* values were presented. SAS version 9.4 (SAS Institute, Cary, NC) was used to conduct the analyses. The trial was registered with ClinicalTrials.gov (NCT02443116) and was overseen by the sponsor's medical monitor and clinical research associate.

Results

Demographics

Between December 5, 2016, and September 5, 2017, 164 patients were screened, and 66 eligible patients were assigned to NGM282 0.3 mg (n = 23), NGM282 1 mg (n = 21), or NGM282 3 mg (n = 22). A total of 63 patients were included in the efficacy analyses: 3 patients in the NGM282 3 mg group did not complete end of treatment assessment due to early

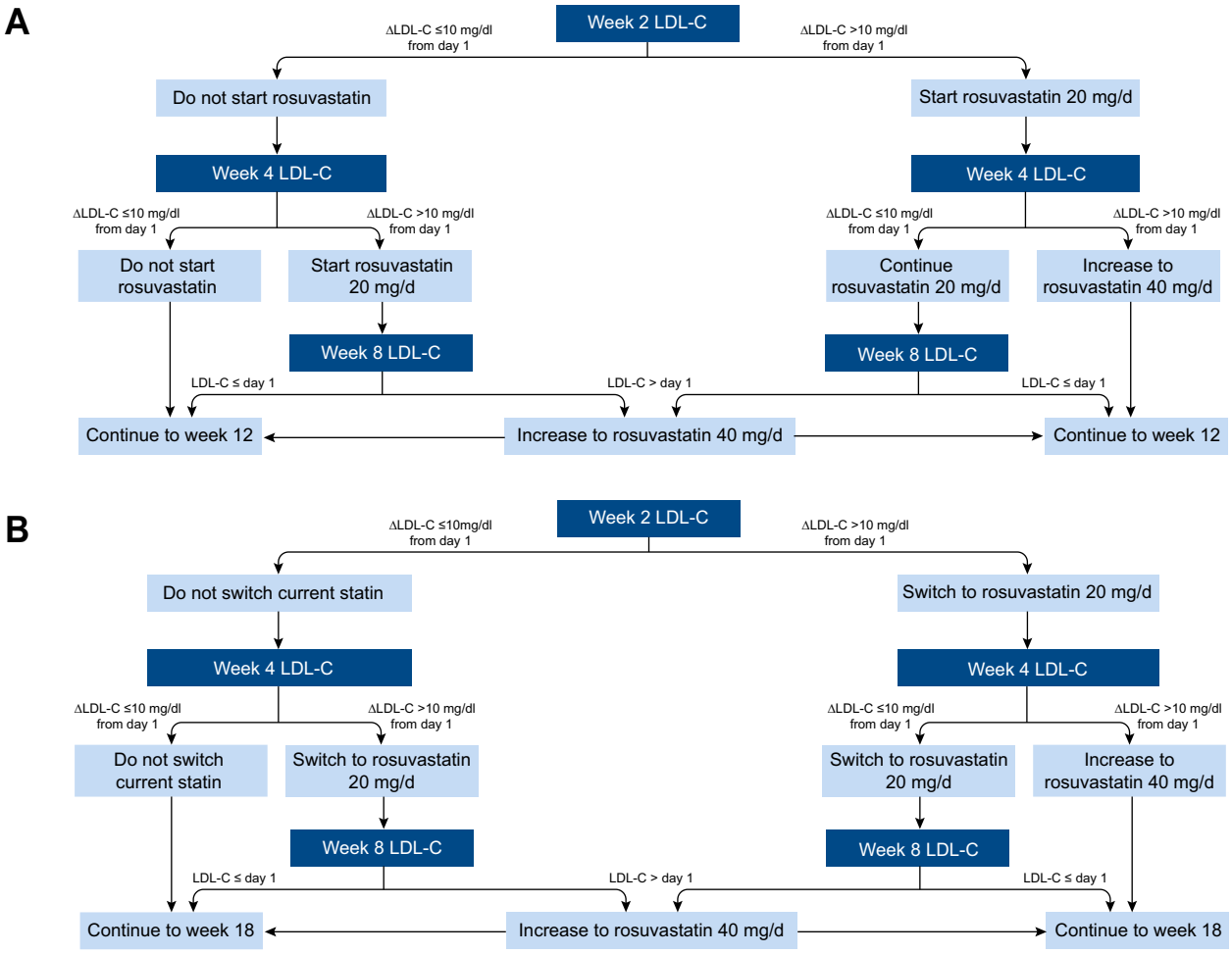

Fig. 1. Rosuvastatin treatment algorithm. (A) Rosuvastatin treatment algorithm in statin-naïve patients. (B) Rosuvastatin treatment algorithm in patients with ongoing statin therapy. Patients received NGM282 at doses of 0.3 mg, 1 mg, or 3 mg per day subcutaneously for 2 weeks, before the biweekly dose escalation of rosuvastatin to a target dose of 40 mg per day began. Both drugs were continued to end of treatment at week 12. LDL-C, low-density lipoprotein cholesterol.

withdrawal and were not included in the efficacy analysis. Baseline demographics and disease characteristics of the 3 dosing groups were similar (Table 1 and Table S2).

Pharmacodynamics

Consistent with the inhibition of the classic pathway of *de novo* bile acid synthesis, treatment with NGM282 resulted in significant reductions in C4, a serum marker of hepatic CYP7A1 activity, indicative of potent target engagement. Reductions in C4 from baseline were significant for all 3 NGM282 dose groups at both week 6 (0.3 mg: −28.0 ng/ml, $p = 0.003$; 1 mg: −23.1 ng/ml, $p < 0.001$; 3 mg: −32.0 ng/ml, $p < 0.001$) and week 12 (0.3 mg: −28.6 ng/ml, $p = 0.004$; 1 mg: −26.7 ng/ml, $p < 0.001$; 3 mg: −33.2 ng/ml, $p < 0.001$) (Table 2 and Fig. 2A). Reductions in serum bile acid levels from baseline were also significant for all NGM282 groups (Table 2 and Fig. 2B).

Impact of NGM282 on lipids and lipoprotein particles

At week 2, LDL-C was significantly elevated with NGM282 0.3 mg (27.6 mg/dl, $p < 0.001$), 1 mg (54.2 mg/dl, $p < 0.001$), and 3 mg (50.6 mg/dl, $p < 0.001$), respectively, compared with baseline values (Table 3 and Fig. 3A). Similar increases in total cholesterol were observed (Table 3 and Fig. 3B). In contrast, HDL-C level remained stable in all 3 NGM282 dose groups at week 2 (Table 3 and Fig. 4A). Triglycerides were decreased with NGM282 1 mg (−34.5 mg/dl, $p < 0.001$) and 3 mg (−47.3 mg/dl, $p < 0.001$), respectively, compared with baseline values, at week 2 (Table 3 and Fig. 4B).

NMR analysis of lipoprotein particles revealed that treatment with NGM282 resulted in significant elevation in concentrations of LDL lipoprotein particles, which was driven mainly by an increase in the large, buoyant LDL particles (194.1 nmol/L, 324.0 nmol/L and 490.8 nmol/L in NGM282 0.3 mg, 1 mg and 3 mg groups, respectively, $p < 0.001$ compared with baseline values for all doses; Table 3 and Fig. S4). Consequently, mean LDL particle size was increased by NGM282 therapy. These changes in LDL particles were accompanied by reductions in the large very low-density lipoprotein (VLDL) particles, with changes of −3.1 nmol/L, −3.7 nmol/L and −4.5 nmol/L in NGM282 0.3 mg ($p = 0.038$), 1 mg ($p < 0.001$) and 3 mg ($p < 0.001$) groups, respectively (Table 3 and Fig. S5). At week 2, mean VLDL particle size was decreased by NGM282 therapy. A decrease in the small, dense HDL particles was observed in the NGM282 1 mg (−2.4 μmol/L, $p = 0.043$) and 3 mg (−4.0 μmol/L, $p < 0.001$) groups (Table 3).

Table 1. Baseline demographics and characteristics.

	NGM282 0.3 mg (n = 23)	NGM282 1 mg (n = 21)	NGM282 3 mg (n = 19)
Age (years)	43.2 (11.4)	51.8 (11.0)	51.4 (12.6)
Male, n (%)	10 (43%)	3 (14%)	4 (21%)
Female, n (%)	13 (56%)	18 (86%)	15 (79%)
Histopathology			
Total NAS score	5.8 (0.8)	5.1 (0.9)	5.7 (1.4)
Fibrosis stage, n (%)			
1	12 (52%)	10 (48%)	3 (16%)
2	3 (13%)	6 (28%)	5 (26%)
3	8 (35%)	5 (24%)	10 (53%)
4	0	0	1 (5%)
Statin status			
Statin-naive	20 (87%)	17 (81%)	13 (68%)
Ongoing statin therapy	3 (13%)	4 (19%)	6 (32%)
Liver fat content by MRI-PDFF			
Liver fat content (%)	20.3 (7.0)	18.9 (6.0)	17.1 (5.6)
Bile acid-related			
C4 (ng/ml)	45.0 (40.6)	31.5 (18.0)	35.0 (24.2)
Serum total bile acids (μmol/L)	4.9 (2.4)	5.1 (2.7)	5.0 (3.1)
Lipids			
Cholesterol (mg/dl)	193.9 (37.4)	205.4 (26.7)	190.1 (36.5)
HDL cholesterol (mg/dl)	41.5 (11.8)	41.1 (8.2)	43.4 (12.2)
LDL cholesterol (mg/dl)	103.4 (29.1)	114.9 (23.5)	96.7 (25.6)
Triglycerides (mg/dl)	165.6 (100.8)	154.5 (46.9)	161.4 (64.6)
Lipoprotein particles			
VLDLP (nmol/L)	51.7 (32.0)	53.5 (18.0)	53.4 (24.1)
Large VLDLP (nmol/L)	8.2 (7.9)	7.3 (4.1)	7.4 (4.9)
Small VLDLP (nmol/L)	23.8 (16.5)	25.8 (11.8)	28.3 (17.0)
VLDLP size (nm)	54.7 (7.6)	53.6 (5.5)	54.5 (5.5)
LDLP (nmol/L)	1,274.4 (340.4)	1,396.0 (221.1)	1,313.0 (328.2)
Large LDLP (nmol/L)	319.8 (205.4)	398.1 (226.0)	280.3 (221.4)
Small LDLP (nmol/L)	802.0 (277.1)	834.3 (233.8)	869.1 (361.6)
LDLP size (nm)	20.5 (0.5)	20.6 (0.6)	20.4 (0.6)
HDLP (μmol/L)	32.3 (6.0)	32.8 (5.1)	31.3 (5.3)
Large HDLP (μmol/L)	6.4 (2.6)	6.1 (2.1)	7.8 (3.2)
Small HDLP (μmol/L)	15.9 (5.9)	20.5 (6.1)	15.9 (6.5)
HDLP size (nm)	9.2 (0.3)	9.1 (0.4)	9.4 (0.4)

Data are mean (SD) or n (%). C4, 7-alpha-hydroxy-4-cholesten-3-one; HDL, high-density lipoprotein; HDLP, high-density lipoprotein particles; LDL, low-density lipoprotein; LDLP, low-density lipoprotein particles; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; SD, standard deviation; VLDLP, very low-density lipoprotein particles.

Impact of rosuvastatin on NGM282-associated changes in lipids and lipoprotein particles

Given the significant elevation in plasma cholesterol concentrations in the NGM282-treated patients at week 2, we implemented a defined LDL-C management algorithm (Fig. 1) to initiate rosuvastatin therapy. Initiation of rosuvastatin therapy in these patients rapidly decreased LDL-C, with changes of −43.2 mg/dl, −59.2 mg/dl and −46.3 mg/dl from week 2 levels for NGM282 0.3, 1, and 3 mg groups, respectively, at week 4 (Fig. 3A). The decline of LDL-C continued through week 6 and week 12 (Tables S3-4). At week 12, patients receiving NGM282 0.3 mg, 1 mg or 3 mg achieved changes in LDL-C from baseline of −29.9 mg/dl (p <0.001), −20.3 mg/dl (p = 0.01) and −14.8 (p = 0.08) mg/dl, respectively (Table 4). The relative changes in LDL-C levels from baseline to week 12 were −28%, −17% and −10% in the NGM282 0.3 mg, 1 mg and 3 mg groups, respectively (Table S5). At week 12, 83% (19/23) of patients receiving NGM282 0.3 mg, 76% (16/21) of patients receiving NGM282 1 mg, and 79% (15/19) of patients receiving NGM282 3 mg achieved LDL-C levels below baseline. A total of 9% (2/23), 28% (6/21) and 32% (6/19) of patients receiving NGM282 0.3 mg, 1 mg and 3 mg, respectively, were on rosuvastatin 40 mg therapy at week 12. Details on rosuvastatin initiation and escalation in these patients are provided (Fig. S1–3).

Initiation of rosuvastatin therapy also rapidly decreased total cholesterol concentrations, with changes of −47.7 mg/dl, −56.8 mg/dl and −51.3 mg/dl from week 2 levels for NGM282 0.3, 1, and 3 mg groups, respectively, at week 4 (Fig. 3B). At week 12, patients receiving NGM282 0.3 mg, 1 mg or 3 mg achieved changes in total cholesterol from baseline of −37.6 mg/dl (p <0.001), −26.5 mg/dl (p = 0.006) and −22.0 mg/dl (p = 0.025), respectively (Table 4). The relative changes in total cholesterol from baseline to week 12 were −18%, −12% and −9% in the NGM282 0.3 mg, 1 mg and 3 mg groups, respectively (Table S5).

At week 12, patients receiving NGM282 1 mg or 3 mg, but not 0.3 mg, had increases in HDL-C from baseline of 4.8 mg/dl (p = 0.022) and 7.2 mg/dl (p <0.001), respectively (Fig. 4A and Table 4). The relative increases in HDL-C levels from baseline to week 12 were 13% and 16% in the NGM282 1 mg and 3 mg groups, respectively (Table S5). Greater increases in HDL-C levels were observed for NGM282 1 mg and 3 mg groups compared with the 0.3 mg group (Table S6).

Plasma concentrations of triglycerides steadily declined over time in all 3 NGM282 dose groups (Fig. 4B and Table 4). At week 12, patients receiving NGM282 0.3 mg, 1 mg or 3 mg achieved further changes in triglycerides from baseline of −37.1 mg/dl (p = 0.03), −44.7 mg/dl (p <0.001) and −60.8 mg/dl (p <0.001), respectively. The relative changes in triglyceride levels from

Table 2. Bile acid-related pharmacodynamic outcomes at week 12.

	Change from baseline to week 12, mean (SD)					
	0.3 mg (n = 23)	*p* value	1 mg (n = 21)	*p* value	3 mg (n = 19)	*p* value
C4						
C4 (ng/ml)	−28.6 (42.7)	0.004	−26.7 (18.3)	<0.001	−33.2 (24.4)	<0.001
Serum bile acids						
Total bile acids (μmol/L)	−1.8 (3.0)	0.008	−3.4 (2.6)	<0.001	−3.5 (2.6)	<0.001
Conjugated primary bile acids						
GCA (μmol/L)	−0.1 (0.2)	0.025	−0.2 (0.2)	<0.001	−0.2 (0.2)	<0.001
TCA (μmol/L)	0.03 (0.17)	0.48	0.03 (0.14)	0.32	−0.02 (0.09)	0.45
GCDCA (μmol/L)	−0.4 (1.2)	0.16	−0.8 (0.9)	<0.001	−0.9 (1.0)	0.002
TCDCA (μmol/L)	0.1 (0.4)	0.12	0.1 (0.1)	0.005	0.1 (0.3)	0.44
Conjugated secondary bile acids						
GDCA (μmol/L)	−0.2 (0.6)	0.044	−0.8 (1.0)	<0.001	−0.6 (0.8)	0.004
TDCA (μmol/L)	0 (0.4)	0.86	−0.2 (0.3)	0.004	−0.2 (0.4)	0.034
GLCA (μmol/L)	−0.01 (0.04)	0.45	−0.02 (0.02)	0.008	−0.02 (0.05)	0.08
TLCA (μmol/L)	0.003 (0.007)	0.08	−0.001 (0.005)	0.19	0.006 (0.033)	0.42
Unconjugated primary bile acids						
CA (μmol/L)	−0.2 (0.5)	0.12	−0.2 (0.3)	0.038	−0.2 (0.4)	0.06
CDCA (μmol/L)	−0.4 (0.6)	0.002	−0.3 (0.3)	<0.001	−0.4 (0.5)	0.001
Unconjugated secondary bile acids						
DCA (μmol/L)	−0.3 (0.3)	<0.001	−0.5 (0.5)	<0.001	−0.6 (0.5)	<0.001
LCA (μmol/L)	0 (0.02)	0.17	−0.02 (0.03)	0.008	−0.02 (0.02)	0.012
UDCA and derivatives						
GUDCA (μmol/L)	−0.2 (0.2)	0.001	−0.3 (0.3)	<0.001	−0.2 (0.2)	0.004
TUDCA (μmol/L)	0 (0.01)	0.26	0 (0.02)	0.41	0.01 (0.02)	0.26
UDCA (μmol/L)	−0.1 (0.2)	0.004	−0.1 (0.2)	0.003	−0.2 (0.3)	0.036

Within each treatment group, the change in continuous outcomes from baseline to week 12 was analyzed using one-sample *t* test. C4, 7-alpha-hydroxy-4-cholesten-3-one; CA, cholic acid; CDCA, chenocholic acid; DCA, deoxycholic acid; GCA, glycocholic acid; GCDCA, glycochenocholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; GUDCA, glycoursodeoxycholic acid; LCA, lithocholic acid; TCA, taurocholic acid; TCDCA, taurochenocholic acid; TDCA, taurodeoxycholic acid; TLCA, taurolithocholic acid; TUDCA, tauroursodeoxycholic acid; UDCA, ursodeoxycholic acid.

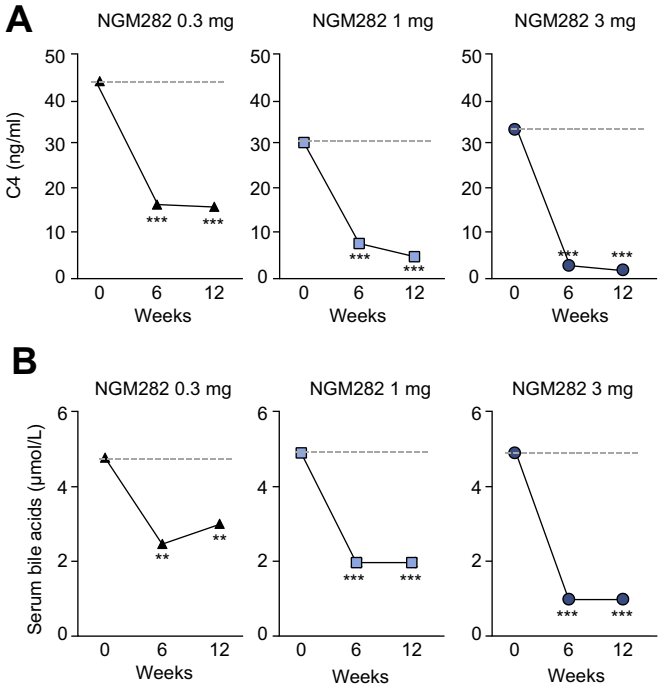

Fig. 2. Markers of target engagement. (A) Serum C4 levels by NGM282 dose. (B) Serum total bile acids levels by NGM282 dose. Serum concentrations of C4 and total bile acids were measured at baseline (week 0), week 6 and week 12 in patients treated with NGM282 0.3 mg, 1 mg, or 3 mg. The dashed line in all panels indicates C4 or total bile acid concentrations at baseline. C4, 7-alpha-cholesten-3-one. ***p <0.001, **p <0.01 *vs.* baseline by one-sample *t* test.

baseline to week 12 were −14%, −26%, −34% in the NGM282 0.3 mg, 1 mg, and 3 mg groups, respectively (Table S5). Greater reductions in triglyceride levels were observed for the NGM282 3 mg group compared with the 0.3 mg group (Table S6).

Moreover, initiation of rosuvastatin therapy rapidly decreased concentrations of LDL particles, and large particle LDL in particular, from week 2 levels (Fig. S4 and Tables S3-4). At week 12, patients receiving NGM282 0.3 mg, 1 mg or 3 mg achieved changes from baseline in LDL particles of −237.9 nmol/L (p <0.001), −170.1 nmol/L (p = 0.013) and −230.3 nmol/L (p = 0.001), respectively, driven mainly by reductions in large LDL particles (Table 4). Decreases in the small, dense LDL particles were noted in the NGM282 3 mg group at week 12. Concentrations of VLDL particles continued to decrease in the NGM282 1 mg and 3 mg groups (Table 4 and Fig. S5). In contrast, increases in HDL particles were observed in all NGM282 dose groups (Table 4 and Fig. S6). Changes in lipids and lipoprotein particles from baseline to week 18, 6 weeks after cessation of NGM282 treatment, are summarized (Table S7).

Impact on liver fat content

At week 12, patients receiving NGM282 0.3 mg, 1 mg or 3 mg attained changes in absolute liver fat content from baseline of −6.5% (p <0.001), −11.0% (p <0.001) and −11.2% (p <0.001), respectively (Table 4). The relative changes in liver fat content from baseline to week 12 were −34%, −57% and −67% in the

Table 3. Effect of NGM282 on lipids and lipoprotein particles at week 2 (prior to rosuvastatin initiation).

	Change from baseline to week 2, mean (SD)					
	0.3 mg (n = 23)	*p* value	1 mg (n = 21)	*p* value	3 mg (n = 19)	*p* value
Lipids						
Triglycerides (mg/dl)	−11.9 (84.9)	0.51	−34.5 (29.8)	<0.001	−47.3 (42.0)	<0.001
Total cholesterol (mg/dl)	26.0 (16.0)	<0.001	47.6 (35.4)	<0.001	42.0 (28.4)	<0.001
HDL-C (mg/dl)	−1.7 (4.7)	0.09	−0.2 (6.7)	0.90	0.6 (5.9)	0.64
LDL-C (mg/dl)	27.6 (19.2)	<0.001	54.2 (31.4)	<0.001	50.6 (30.1)	<0.001
Lipoprotein particles						
VLDLP (nmol/L)	4.5 (17.8)	0.27	−4.7 (21.1)	0.34	−11.0 (27.1)	0.08
Large VLDLP (nmol/L)	−3.1 (6.2)	0.038	−3.7 (3.1)	<0.001	−4.5 (4.0)	<0.001
Small VLDLP (nmol/L)	5.9 (21.8)	0.24	6.2 (18.7)	0.16	1.4 (24.9)	0.80
VLDLP size (nm)	−5.7 (10.2)	0.022	−6.6 (7.5)	0.001	−8.3 (6.8)	<0.001
LDLP (nmol/L)	313.0 (185.0)	<0.001	526.6 (335.4)	<0.001	414.0 (325.8)	<0.001
Large LDLP (nmol/L)	194.1 (139.6)	<0.001	324.0 (189.2)	<0.001	490.8 (244.4)	<0.001
Small LDLP (nmol/L)	50.0 (224.8)	0.33	101.8 (243.0)	0.08	−94.1 (312.8)	0.18
LDLP size (nm)	0.3 (0.3)	<0.001	0.5 (0.4)	<0.001	0.7 (0.5)	<0.001
HDLP (μmol/L)	−1.1 (2.7)	0.10	−0.7 (3.4)	0.41	−2.5 (3.8)	0.007
Large HDLP (μmol/L)	−0.5 (1.2)	0.11	−0.3 (1.8)	0.41	−0.7 (2.1)	0.17
Small HDLP (μmol/L)	−0.1 (5.5)	0.93	−2.4 (4.9)	0.043	−4.0 (4.3)	<0.001
HDLP size (nm)	0 (0.2)	1.00	0 (0.3)	0.94	0 (0.4)	0.90

Within each treatment group, the change in continuous outcomes from baseline to week 2 was analyzed using one-sample *t* test. HDL-C, high-density lipoprotein cholesterol; HDLP, high-density lipoprotein particles; LDL-C, low-density lipoprotein cholesterol; LDLP, low-density lipoprotein particles; SD, standard deviation; VLDLP, very low-density lipoprotein particles.

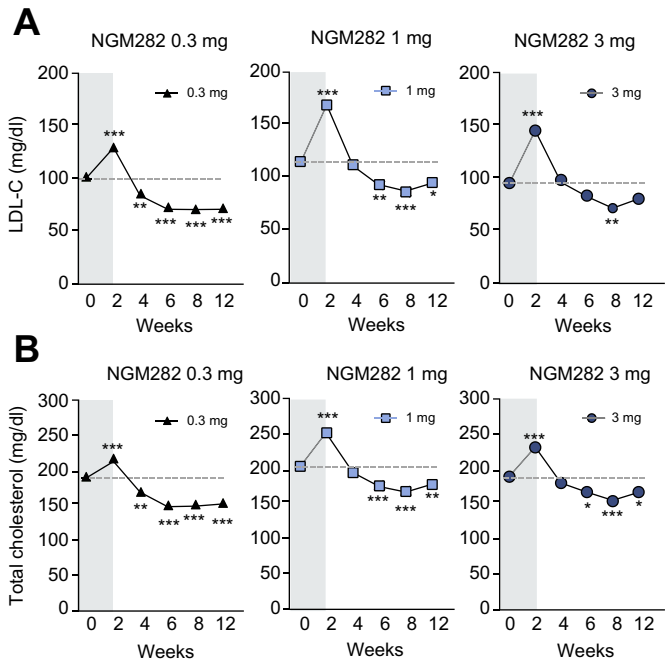

Fig. 3. Levels of LDL-C and total cholesterol over time. (A) LDL-C levels over time. (B) Total cholesterol levels over time. Concentrations of LDL-C and total cholesterol were measured at baseline (week 0), weeks 2, 4, 6, 8, and 12 in patients treated with NGM282 0.3 mg, 1 mg, or 3 mg. The dashed line in all panels indicates serum LDL-C or total cholesterol concentrations at baseline. The shaded area in all panels represents the period of NGM282 therapy before rosuvastatin commencement. LDL-C, low-density lipoprotein cholesterol. ***p <0.001, **p <0.01, *p <0.05 *vs.* baseline by one-sample *t* test.

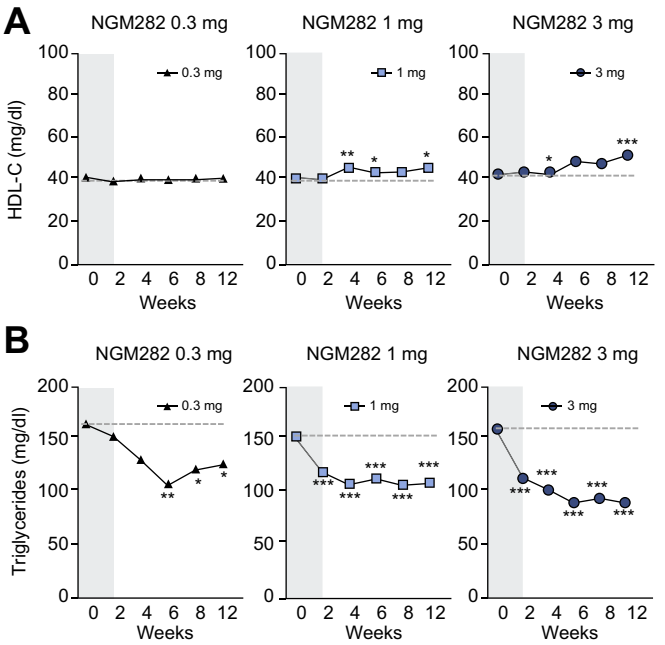

Fig. 4. Levels of HDL-C and triglycerides over time. (A) HDL-C levels over time. (B) Triglycerides levels over time. Concentrations of HDL-C and triglycerides were measured at baseline (week 0), weeks 2, 4, 6, 8, and 12 in patients treated with NGM282 0.3 mg, 1 mg, or 3 mg. The dashed line in all panels indicates serum HDL-C or triglycerides concentrations at baseline. The shaded area in all panels represents the period of NGM282 therapy before rosuvastatin commencement. HDL-C, high-density lipoprotein cholesterol. ***p <0.001, **p <0.01, *p <0.05 *vs.* baseline by one-sample *t* test.

NGM282 0.3 mg, 1 mg and 3 mg groups, respectively. At week 12, 48% (11/23) of patients receiving NGM282 0.3 mg, 81% (17/21) of patients receiving NGM282 1 mg, and 100% (19/19) of patients receiving NGM282 3 mg achieved ≥30% reduction in liver fat content from baseline. The magnitude and response rate in liver fat reduction are similar to the previously reported double-blind, placebo-controlled trial of NGM282,[12] suggesting that co-administration of rosuvastatin did not attenuate the efficacy of NGM282 on steatosis reduction.

Safety

Safety and tolerability of NGM282 were similar to those previously reported in patients with NASH.[12] No new adverse signals

Table 4. Key outcomes at week 12 (end of treatment).

	Change from baseline to week 12, mean (SD)					
	0.3 mg (n = 23)	*p* value	1 mg (n = 21)	*p* value	3 mg (n = 19)	*p* value
Lipids						
Triglycerides (mg/dl)	−37.1 (76.8)	0.030	−44.7 (41.1)	<0.001	−60.8 (50.7)	<0.001
Total Cholesterol (mg/dl)	−37.6 (31.2)	<0.001	−26.5 (38.8)	0.006	−22.0 (39.1)	0.025
HDL-C (mg/dl)	−0.5 (7.0)	0.75	4.8 (8.7)	0.022	7.2 (7.5)	<0.001
LDL-C (mg/dl)	−29.9 (26.2)	<0.001	−20.3 (33.1)	0.013	−14.8 (34.7)	0.08
Lipoprotein particles						
VLDLP (nmol/L)	−4.6 (24.8)	0.38	−19.7 (17.8)	<0.001	−22.4 (21.6)	<0.001
Large VLDLP (nmol/L)	−2.8 (6.5)	0.05	−3.7 (3.8)	<0.001	−4.8 (4.4)	<0.001
Small VLDLP (nmol/L)	1.7 (19.1)	0.67	−6.5 (19.6)	0.15	−9.1 (20.2)	0.06
VLDLP size (nm)	−1.4 (8.1)	0.40	−0.9 (6.4)	0.52	−3.9 (7.1)	0.027
LDLP (nmol/L)	−237.9 (231.3)	<0.001	−170.1 (278.5)	0.013	−230.3 (348.2)	0.001
Large LDLP (nmol/L)	−121.3 (167.2)	0.002	−139.0 (296.3)	0.05	−72.6 (299.4)	0.30
Small LDLP (nmol/L)	−61.4 (204.1)	0.16	13.2 (237.8)	0.80	−151.3 (307.0)	0.046
LDLP size (nm)	−0.3 (0.5)	0.008	−0.2 (0.6)	0.12	−0.2 (0.6)	0.29
HDLP (μmol/L)	1.6 (3.6)	0.039	3.2 (6.4)	0.037	3.3 (3.9)	0.002
Large HDLP (μmol/L)	0.2 (1.8)	0.68	0.6 (2.7)	0.37	1.6 (2.6)	0.014
Small HDLP (μmol/L)	0.8 (7.6)	0.63	−1.9 (6.3)	0.19	−2.3 (5.2)	0.06
HDLP size (nm)	0.1 (0.2)	0.21	0.2 (0.4)	0.09	0.3 (0.5)	0.021
Imaging by MRI-PDFF						
Absolute liver fat content (%)	−6.5 (4.6)	<0.001	−11.0 (5.0)	<0.001	−11.2 (4.2)	<0.001
Relative liver fat content (%)	−34.5 (25.3)	<0.001	−56.8 (19.0)	<0.001	−66.6 (17.1)	<0.001

Within each treatment group, the change in continuous outcomes from baseline to week 12 was analyzed using one-sample *t* test. HDL-C, high-density lipoprotein cholesterol; HDLP, high-density lipoprotein particles; LDL-C, low-density lipoprotein cholesterol; LDLP, low-density lipoprotein particles; SD, standard deviation; VLDLP, very low-density lipoprotein particles.

were reported in the current study. Of note, rosuvastatin was well-tolerated in patients treated with NGM282. Among the 63 patients who initiated rosuvastatin therapy during the study, none reported muscle pain or rhabdomyolysis, adverse events previously associated with statin use.

Discussion

We have previously shown that NGM282 produced rapid and sustained improvements in liver fat content, aminotransferases, and non-invasive serum fibrosis biomarkers over a 12-week treatment period in patients with NASH.[12] In this open-label, multicenter study, we showed that significant decreases in serum levels of C4, a marker of hepatic CYP7A1 activity, and ensuing increases in total cholesterol and LDL-C were observed with NGM282 treatment. NGM282-associated elevation of cholesterol is mainly driven by an increase in the large, buoyant LDL particles, which are considered to be less atherogenic than the small, dense LDL particles.[17] Importantly, co-administration of rosuvastatin in these patients reduced concentrations of total cholesterol, LDL-C and LDL particles to levels below baseline. Furthermore, NGM282 in combination with rosuvastatin for 12 weeks resulted in significant reductions in triglycerides and VLDL particles, as well as increases in HDL-C and HDL particles. Liver fat content was reduced by 28%, 57% and 67% from baseline to the end of treatment in the NGM282 0.3 mg, 1 mg and 3 mg groups, respectively.

NGM282 reduces bile acid synthesis by blocking the conversion of cholesterol to bile acids, a major mechanism of cholesterol disposal.[18] Although not exclusive of other mechanisms, inhibiting the conversion of cholesterol to bile acids could increase serum cholesterol levels. Indeed, in a double-blind, randomized, placebo-controlled study in patients with biopsy-proven NASH,[12] showed that a reduction in serum levels of C4, a surrogate of bile acid synthesis, was the laboratory parameter most strongly correlated with elevated LDL-C (Spearman correlation, r = −0.53; p <0.001; Table S8). Furthermore, changes in liver fat content were highly correlated with changes in C4 (r = 0.53; p <0.001) and LDL-C (r = −0.54; p <0.001), consistent with the mechanism of action of NGM282.[12] Indeed, obeticholic acid, an agonist of the farnesoid X receptor that induces FGF19 secretion, was previously shown to be associated with elevated LDL-C.[19] Although the NGM282-associated cholesterol increase was mainly driven by the large, buoyant LDL particles that are thought to be less atherogenic than the small, dense LDL particles,[17] its impact on cardiovascular risk is not yet clear. Thus, it is important to explore the use of concomitant lipid-lowering therapies to support chronic administration of NGM282.

HMG-CoA reductase inhibitors (statins) have long been recognized as an effective approach to reduce total cholesterol, LDL-C and the risk of cardiovascular events, such as myocardial infarction and stroke.[20] A 2010 meta-analysis of 26 randomized clinical trials involving close to 170,000 participants confirmed the benefit of LDL-C lowering with statins.[21] While this meta-analysis showed that a 38 mg/dl reduction in LDL-C can result in a 21% decrease in major vascular events, a 19% reduction in coronary revascularizations, and a 16% reduction in cerebrovascular accident, agents that improve clinical outcomes despite raising LDL-C have also been reported, largely due to improving the underlying diseases.[22–23] Although reluctance to use statins by some providers in the setting of liver disease persists due to a concern for drug-induced liver injury,[24] multiple studies have demonstrated the safety of statins in patients with liver diseases, including NASH.[25,26] Consequently, EASL-EASD-EASO recommend that statins may be used to manage dyslipidemia in the current clinical practice guidelines.[27]

The JUPITER trial demonstrated that rosuvastatin can improve cardiovascular outcome by reducing the incidence of myocardial infarction, stroke and death from cardiovascular cause, without increasing myopathy.[28] Furthermore, the generic formulation of rosuvastatin was recently approved in the US. To

provide patients with a statin that is supported by the best available evidence of cardiovascular benefit while being cost-effective, we elected to use rosuvastatin in our trial. To optimize lipid management, all enrolled patients underwent a stepwise, incremental dose titration of rosuvastatin and were managed according to the study algorithm. Higher doses of NGM282 were associated with dose escalation of rosuvastatin. Nevertheless, at week 12, reductions from baseline in the level of total cholesterol of up to 18% (or −38 mg/dl) and LDL-C of up to 28% (or −30 mg/dl) were observed in NGM282-treated patients. Significant reductions in LDL particle numbers were achieved in all NGM282 dose groups. Importantly, rosuvastatin was safe and well-tolerated when co-administered with NGM282 in these patients.

The pronounced reduction (up to 34% in 12 weeks) in plasma triglycerides by NGM282 is particularly noteworthy, given that rosuvastatin was reported to only marginally reduce triglycerides (17% after 12-month of treatment) in the JUPITER trial.[28] Plasma triglyceride levels reflect the balance between production and clearance of triglyceride-rich lipoproteins. The reduction in triglyceride is consistent with our previous study showing that NGM282 reduces lipogenesis by suppressing the expression of key genes, including fatty acid synthetase, ATP citrate lyase, acetyl-Coenzyme A carboxylase beta, stearoyl-Coenzyme A desaturase 1, diacylglycerol O-acyltransferase 2, ELOVL family member 6 and elongation of long chain fatty acids, in animal models.[29] Both the number and size of triglyceride-rich, large VLDL lipoprotein particles was rapidly reduced by NGM282 therapy at week 2. Once secreted from the liver, VLDL particles undergo lipoprotein lipase-mediated lipolysis in the blood to form LDL particles.[30] Future research should examine whether the increased LDL particles observed in NGM282-treated patients derive from the catabolism and clearance of VLDL.

Multiple factors must be considered in cardiovascular risk assessment of NGM282 therapy in NASH. First, the presence and severity of NAFLD independently predict fatal and non-fatal cardiovascular events.[31] NAFLD is associated with subclinical coronary atherosclerosis, and non-calcified plaque formation in particular.[32] There is now accumulating evidence that NAFLD not only accelerates coronary atherosclerosis, but also promotes left ventricular hypertrophy, cardiac valvular calcification, cardiac arrhythmia and cardiac dysfunction.[33] Administration of NGM282 resulted in a rapid and profound reduction in liver fat content,[12] which may improve risks associated with a fatty liver. Second, inflammation is a strong risk factor for cardiovascular disease. Individuals with chronic inflammatory conditions, such as rheumatoid arthritis, systemic lupus erythematous or viral infection, have an increased risk of ASCVD.[34,35] Recent trials have shown that anti-inflammatory therapy targeting the interleukin-1β pathway with canakinumab led to a significantly lower rate of cardiovascular events than placebo, independent of lipid-lowering effects.[36] It is intriguing that while the eradication of hepatitis C virus reduces cardiovascular events and overall mortality,[23] a rapid elevation of LDL-C as high as +40 mg/dl within 4–6 weeks of antiviral therapy was reported.[37] Treatment with NGM282 reduces the hepatic expression of interleukin-1β and numerous pro-inflammatory cytokines/chemokines in animal models of NASH,[29] and markers of hepatic inflammation, such as alanine aminotransferase and aspartate aminotransferase, in patients with NASH.[12] Consequently, NGM282 may reduce cardiovascular risk by attenuating hepatic and systemic inflammation. Third, increasing evidence also points to bile acids as an important risk factor for cardiovascular disease. Bile acids can directly impair cardiac mitochondrial function and induce cardiomyopathy in animal models.[38,39] Serum bile acid levels are elevated in advanced liver disease, are correlated with disease severity, and are associated with structural cardiac dysfunction in patients with cirrhosis.[40,41] NGM282 potently inhibits *de novo* bile acid synthesis through suppression of CYP7A1, significantly reducing serum levels of bile acids, and thereby potentially affecting bile acid-related cardiovascular complications. Fourth, the atherogenic potential of lipoproteins is complex and is strongly associated with the number and size of lipoprotein particles.[42] Patients with NASH exhibit an atherogenic lipoprotein profile, with increased size and concentrations of VLDL particles and increased concentrations of LDL particles, particularly small dense LDL particles, compared with control individuals.[16] Administration of NGM282 rapidly reduced the size and concentrations of large VLDL particles at week 2, consistent with a less atherogenic VLDL profile. At week 2, the NGM282-induced increase in LDL is mainly driven by the large LDL particles, similar to that observed with the insulin sensitizer pioglitazone.[43] Nonetheless, co-administration of rosuvastatin reduced LDL-C and concentrations of LDL particles to levels below baseline in NGM282-treated patients at week 12. The overall lipoprotein profile (reductions in concentrations of LDL and VLDL particles, and an increase in HDL particles) at the end of treatment supports an anti-atherogenic potential of treatment with NGM282 in combination with rosuvastatin in patients with NASH. Lastly, the dyslipidemic triad (high triglyceride, low HDL-C, and high small dense LDL-C) typical of NASH is also an independent risk factor for cardiovascular events. The ratio of triglyceride and HDL-C in particular, is a surrogate marker for insulin resistance and associated with carotid atherosclerosis.[44] NGM282 decreases triglycerides and increases HDL-C, thus improving dyslipidemia and possibly associated cardiovascular risk.

Limitations of our trial included the lack of a placebo group, as well as the lack of blinding with regard to treatment and randomization. Although participants were aware of the intervention due to its open-label design, the execution of the LDL-C algorithm prevented physicians from influencing the decision about when to start treatment with statin. The selection of a threshold of 10 mg/dl elevation in LDL-C to trigger statin initiation may underestimate treatment effect, especially in patients with lower baseline LDL-C. Despite its invasive nature, liver biopsy was still required for enrollment in this trial. With continued research, decreasing cost, greater availability and wider acceptance of imaging and biochemical measures, future trials in NASH will likely not require a biopsy for trial inclusion, as risk prediction algorithms and biomarkers become more accurate. Additional limitations of this study include a relatively short treatment period, small overall number of patients, and the lack of smoking history in baseline data collection, which precluded calculation of the ASCVD risk score or Framingham Risk Assessment Tool for estimating 10-year risk of a coronary event.

Future trials should include longer term follow-up in larger cohorts of NASH patients with double-blind, placebo-controlled design, with statin/placebo over-encapsulation to mitigate any source of bias. Imaging tools such as intravascular ultrasonography and computed tomography or MRI angiography may be utilized to measure progression as well as regression of atherosclerosis. Future studies should also explore

lower rosuvastatin doses, slower escalation and alternative lipid-lowering therapies for co-administration with NGM282. Further understanding of the cardiovascular effects of NASH and insights into potential cardiovascular benefits of treating this chronic disease may have important implications on clinical practice.

Conclusions

The LDL-C elevation associated with NGM282 administration was effectively managed with statin therapy. Co-administration of NGM282 and rosuvastatin reduces plasma triglycerides, total cholesterol, LDL-C, and increases HDL-C in patients with NASH.

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Conflict of interests

MER reports research/grant support from Novartis and being on advisory committees or review panels for Intercept, Abbvie; consulting for Intercept, Novartis, NGM Bio, Shire, Enanta, Madrigal, Gilead, Immuron, Taiwan J, Fibrogen and Pfizer. MFA reports grant/research support from NIH/NIDDK, Allergan, Conatus, Galectin, Galmed, Genfit, Gilead, Immuron, Intercept, Madrigal, Shire, NGM Bio, BMS, Excelenz, Boehringer Ingelheim, Enanta, Prometheus and TaiwanJ; is on Speakers' Bureaus for Alexion; is a consulting advisor for Allergan, TaiwanJ, NGM Bio, BMS, Lexicon, MedImmune and Pfizer. MAC reports being employee and stockholder of LabCorp. LL, SJR, and AMD report being employees and stockholders of NGM Bio. SAH reports grant/research support from Allergan, Conatus, Galectin, Galmed, Genfit, Gilead, Immuron, Intercept, Madrigal, NGM Bio, TaiwanJ and Cymabay; participate on Speakers' Bureaus for Alexion and Abbvie; is a consulting advisor for Prometheus, Chronic Liver Disease Foundation, Cirius, Echosens, Cymabay, Ascella, Genfit, Gilead, Intercept, Madrigal, NGM Bio, Novartis, Perspectum, Corcept, Innovate, Akero, CiVi, Second Genome and Metacrine. All other authors declare no conflict of interest.

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

Authors' contributions

MER, MFA, SJR, AMD, and SAH participated in study design. MER, JFT, MFA, AHP, MAC, and SAH were responsible for data collection. MER, MJJ, LL, SJR participated in data analysis. MER, JFT, MFA, MAC, LL, SJR, AMD, and SAH participated in data interpretation. All authors participated in manuscript review and writing. MER, LL, SJR were responsible for preparation of the tables and figures.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2018.11.032.

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Author names in bold designate shared co-first authorship

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