TVB-2640 (FASN Inhibitor) for the Treatment of Nonalcoholic Steatohepatitis: FASCINATE-1, a Randomized, Placebo-Controlled Phase 2a Trial

Rohit Loomba, Rizwana Mohseni, K. Jean Lucas, Julio A Gutierrez, Robert G Perry, James F Trotter, Robert S Rahimi, Stephen A Harrison, Veeral Ajmera, Jeffrey D Wayne, et al.

Gastroenterology 2021;■:1–12

TVB-2640 (FASN Inhibitor) for the Treatment of Nonalcoholic Steatohepatitis: FASCINATE-1, a Randomized, Placebo-Controlled Phase 2a Trial

Rohit Loomba,[1] Rizwana Mohseni,[2] K. Jean Lucas,[3] Julio A. Gutierrez,[4] Robert G. Perry,[5] James F. Trotter,[6] Robert S. Rahimi,[6] Stephen A. Harrison,[7] Veeral Ajmera,[1] Jeffrey D. Wayne,[8] Marie O'Farrell,[9] William McCulloch,[9] Katharine Grimmer,[9] Mary Rinella,[10] Vincent Wai-Sun Wong,[11] Vlad Ratziu,[12] Gregory J. Gores,[13] Brent A. Neuschwander-Tetri,[14] and George Kemble[9]

[1]Nonalcoholic Fatty Liver Disease Research Center, Division of Gastroenterology and Hepatology, Department of Medicine, University of California at San Diego, La Jolla, California; [2]Catalina Research Institute, Montclair, California; [3]Lucas Research, Morehead City, North Carolina; [4]Prosciento, Chula Vista, California; [5]Panax Clinical Research, Miami Lakes, Florida; [6]Baylor University Medical Center, Texas Digestive Disease Consultants, Dallas, Texas; [7]Pinnacle Clinical Research, San Antonio, Texas; [8]Clinical Trials Research, Lincoln, California; [9]Sagimet Biosciences Inc., San Mateo, California; [10]Feinberg School of Medicine, Northwestern University, Chicago, Illinois; [11]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong; [12]Sorbonne Université, Assistance Publique-Hôpitaux de Paris, Hôpital Pitié-Salpêtriére, Institute for Cardiometabolism and Nutrition, Paris, France; [13]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Mayo Clinic, Rochester, Minnesota; and [14]Division of Gastroenterology and Hepatology, Saint Louis University School of Medicine, St Louis, Missouri

BACKGROUND & AIMS: Increased de novo lipogenesis creates excess intrahepatic fat and lipotoxins, propagating liver damage in nonalcoholic steatohepatitis. TVB-2640, a fatty acid synthase inhibitor, was designed to reduce excess liver fat and directly inhibit inflammatory and fibrogenic pathways. We assessed the safety and efficacy of TVB-2640 in patients with nonalcoholic steatohepatitis in the United States. **METHODS:** 3V2640-CLIN-005 (FASCINATE-1) was a randomized, placebo-controlled, single-blind study at 10 US sites. Adults with ≥8% liver fat, assessed by magnetic resonance imaging proton density fat fraction, and evidence of liver fibrosis by magnetic resonance elastography ≥2.5 kPa or liver biopsy were eligible. Ninety-nine patients were randomized to receive placebo or 25 mg or 50 mg of TVB-2640 (orally, once-daily for 12 weeks). The primary end points of this study were safety and relative change in liver fat after treatment. **RESULTS:** Liver fat increased in the placebo cohort by 4.5% relative to baseline; in contrast TVB-2640 reduced liver fat by 9.6% in the 25-mg cohort (n = 30; least squares mean: −15.5%; 95% confidence interval, −31.3 to −0.23; $P = .053$), and 28.1% in the 50-mg cohort (n = 28; least squares mean: −28.0%; 95% confidence interval, −44.5 to −11.6; $P = .001$). Eleven percent of patients in the placebo group achieved a ≥30% relative reduction of liver fat compared to 23% in the 25-mg group, and 61% in the 50-mg group ($P < .001$). Secondary analyses showed improvements of metabolic, pro-inflammatory and fibrotic markers. TVB-2640 was well tolerated; adverse events were mostly mild and balanced among the groups. **CONCLUSIONS:** TVB-2640 significantly reduced liver fat and improved biochemical, inflammatory, and fibrotic biomarkers after 12 weeks, in a dose-dependent manner in patients with nonalcoholic steatohepatitis. ClinicalTrials.gov, Number NCT03938246.

Keywords: Palmitate; PRO-C3.

Nonalcoholic fatty liver disease (NAFLD) encompasses a progressive, histologically recognizable range of liver disease from simple steatosis to nonalcoholic steatohepatitis (NASH) without fibrosis, NASH with fibrosis, and, ultimately, cirrhosis. The risk of mortality increases along the histologic spectrum of disease and is significantly higher than the general population controls at all stages.[1] The global prevalence of NAFLD, estimated at 24% in 2017, is expected to increase along with rates of obesity and type 2 diabetes (T2D).[2] This will drive pressure on health care systems by increasing the burden of patients with liver disease fueling the ongoing rise in NASH as an indication for liver transplantation, cardiovascular disease, and both hepatocellular and extrahepatic cancers. There are currently no approved therapeutics in the United States or European Union for this emerging epidemic and limited options for improving their hepatic and cardiovascular health.[3]

Increased intrahepatic fat and the generation of lipotoxic metabolites initiate and drive the progression of NAFLD by damaging hepatocytes, stimulating inflammatory responses,

Abbreviations used in this paper: **ACC, acetyl-CoA-carboxylase; ALT, alanine aminotransferase; CK18, cytokeratin-18; DAG, diacylglycerol; DNL, de novo lipogenesis; FASN, fatty acid synthase; FGF21, fibroblast growth factor-21; HDL-chol, high-density lipoprotein cholesterol; LDL-chol, low-density lipoprotein cholesterol; MRI-PDFF, magnetic resonance imaging proton density fat fraction; NAFLD, Nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PIIINP, plasma N-terminal propeptide of type III procollagen; T2D, type 2 diabetes; TEAE, treatment-emergent adverse event; TG, triglyceride; TIMP1, tissue inhibitor of metalloproteinase 1; Tot-chol, total cholesterol; UHPLC-MS, ultra-high-performance liquid chromatography.**

0016-5085
https://doi.org/10.1053/j.gastro.2021.07.025

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

The enzyme fatty acid synthase is responsible for increased lipogenesis and the excess levels of hepatic fat and lipotoxins driving liver damage in patients with nonalcoholic steatohepatitis (NASH).

NEW FINDINGS

Administration of TVB-2640, a fatty acid synthase inhibitor, resulted in a ≥30% reduction of liver fat relative to baseline in 61% of patients. Improvements of biomarkers of hepatocyte damage, metabolism, lipotoxicity, inflammation, and fibrosis were also observed.

LIMITATIONS

The short duration and lack of liver biopsy did not allow for direct observation of liver tissue improvement. The single-blind design did not eliminate the potential for some bias in patient counseling.

IMPACT

TVB-2640 produced positive effects on multiple parameters of liver damage in patients with NASH. These results support further development of TVB-2640 for the treatment of this disease.

and activating profibrotic stellate cells in the liver.[4–6] The de novo lipogenesis (DNL) pathway, in which the enzymes acetyl-CoA-carboxylase (ACC) and fatty acid synthase (FASN) convert metabolites of simple dietary sugars into the fatty acid palmitate, plays a major role in creating excess fat in the liver and generating certain lipotoxic molecules. Insulin resistance and blood glucose may further stimulate DNL in NAFLD patients resulting in increased intrahepatic triglyceride levels.[7]

Lipotoxicity is a major contributor to the pathogenic mechanisms driving the progressive nature of NASH. Palmitate itself can directly cause liver injury and NASH in experimental models.[8] This saturated fatty acid directly activates the Nod-like receptor family pyrin domain containing 3 inflammasome in inflammatory cells and in hepatic stellate cells.[9,10] In addition, palmitate is a building block for other pro-inflammatory and pro-fibrotic lipotoxins, including certain ceramides, sphingomyelins, and diacylglycerols (DAGs). NASH patients have increased levels of ceramides that promote insulin resistance, inflammation, and production of reactive oxygen species.[11,12] In addition to ceramides, specific DAGs, especially with palmitate acyl chain, potently activate protein kinase C isoforms in liver and muscle, which blunts the peripheral and hepatic insulin responses.[13]

Adult mice in which the FASN gene was genetically inactivated in the liver showed no discernable differences from their wild-type littermates, particularly there were no alterations of metabolic markers, including plasma triglycerides or liver tissues.[14] A paradoxical result was observed in that significant hepatic steatosis developed when these FASN knockout animals were fed a laboratory derived zero-fat diet lacking all sources of fat. Suprapharmacologic doses of TVB-2640 did not cause hepatic steatosis, even in animals dosed daily from 4 to 39 weeks. Preclinical studies validated that FASN inhibition not only reduces intrahepatic fat, but also inhibits diet-induced inflammation and insulin resistance in murine NASH models.[15] Ex vivo studies have shown that FASN inhibition reduces fibrogenesis in human hepatic stellate cells.[16]

TVB-2640 is a selective, potent, reversible inhibitor of human FASN enzymatic activity. This once-daily oral compound has been evaluated in more than 200 human subjects. We established the mechanism of action of TVB-2640 in a Phase 1 clinical study.[17] DNL was measured in healthy adult subjects with metabolic comorbidities by measuring conversion of [^{13}C]-acetate to labeled palmitate before and after 10 daily doses of 50 mg, 100 mg, or 150 mg of TVB-2640. DNL was reduced at all doses and in a dose-dependent manner; 150 mg resulted in nearly complete inhibition. Based on results of these preclinical and clinical studies, we designed this randomized, placebo-controlled phase 2 study (FASCINATE-1) to assess safety and test the effect of TVB-2640 on liver fat and markers of liver and metabolic health in NASH patients.

Methods

Study Design and Participants

This randomized, multicenter placebo-controlled, single-blinded clinical study, 3V2640-CLIN-005, FASCINATE-1, enrolled subjects with clinical or histologic evidence of NASH (ClinicalTrials.gov, Number NCT03938246). This study was performed in accordance with ethical principles of the Declaration of Helsinki and consistent with the International Conference on Harmonization, Good Clinical Practice, and applicable regulatory requirements.

The study population included male and female subjects aged 18 years and older with magnetic resonance imaging proton density fat fraction (MRI-PDFF) ≥8% and either biopsy-proven NASH within 2 years before randomization or magnetic resonance elastography ≥2.5 kPa during screening to sample a population of patients with NASH and fibrosis.[18] Women of childbearing potential were allowed to enroll after a negative test for pregnancy and agreement to maintain appropriate contraception during the study and for 3 months after the final dose. All patients were instructed to take their dose with food in the morning from days 1 to 8 and evenings with food thereafter. Forty-nine patients were enrolled at 10 sites in the United States between April and July 2019 and dosed with either 25 mg TVB-2640 or placebo daily for 12 weeks (randomized 2:1). After completion of this first cohort, a Safety Review Committee composed of independent hepatology, ophthalmology, and dermatology experts reviewed adverse events, including any eye and skin reports, vital signs, electrocardiogram data, and laboratory values of interest. The Safety Review Committee recommended escalation of TVB-2640 to the planned 50-mg dose. Fifty patients were enrolled between December 2019 and February 2020 and dosed with either 50 mg TVB-2640 or placebo daily (randomized 2:1). Five extended their dosing period and end of dosing MRI-PDFF beyond 12 weeks due to the impact of COVID-19 restrictions on study visits. Patients on stable diabetes-modifying agents or

statins for 3 months or more were allowed to enroll in the study and remain on their prior medications.

Randomization and Blinding

At each dose, subjects were randomly assigned to TVB-2640 or placebo at a 2:1 ratio, with randomization stratified by T2D status. The randomization system used was the IBM Randomization and Trial Supply Management System. A single-blind randomization list was created by the study statistician and per-protocol eligible subjects were entered into the system at the site level. The IBM wizard system interface used the defined stratification variable (T2D) and randomly assigned the subject to active or placebo in a 2:1 ratio. Study and site staff were assigned access based on role on study as defined by the site-specific delegation logs. The IBM system followed Good Clinical Practice and is Part 11-compliant. Personnel responsible for reading and interpreting MRI-PDFF images were blinded to the subjects' treatment assignment group, as were laboratories conducting biomarker assays. Study patients were blinded; all other study personnel were unblinded.

Procedures

Subjects had an MRI-PDFF measured before the start of treatment (predose), at the end of the initial 12-week treatment period and at week 16, four weeks after completing the treatment period. During the study, safety was assessed by vital signs (oral temperature, pulse, respiratory rate, and blood pressure); 12-lead electrocardiogram; physical examination, including ophthalmologic examination if necessary; and clinical laboratory testing (hematology, chemistry, and urinalysis). Upper limit of normal values for alanine aminotransferase (ALT) were 41 U/L for male subjects and 33 U/L for female subjects according to the central laboratory. Blood samples for pharmacokinetics were collected from a portion of subjects in each TVB-2640 cohort at 2, 4, 6, and 24 hours after the first dose and immediately predose and at 2, 4, and 6 hours post dose at week 2. Bioanalytical analysis using a validated liquid chromatography-mass spectrometry method and pharmacokinetic calculations were performed by Alturas.

Lipidomic analyses were performed at One Way Liver, S.L. (Derio, Spain) by ultra-high-performance liquid chromatography (UHPLC-MS) analyzing methanol and chloroform/methanol extracts.[19] For metabolites within their corresponding linear detection range, univariate statistical analysis was conducted using the Wilcoxon rank test for the comparisons. Tripalmitin was quantitated by using a calibration curve prepared from a stock standard solution of tripalmitin (TG 16:0/16:0/16:0) (Larodan, Sweden). Patient plasma extracts were mixed with sodium chloride (50 mM) and chloroform/methanol (2:1) in 1.5 mL microtubes on ice, vortexed, incubated for 1 hour at –20°C and centrifuged. The organic phase was dried, reconstituted in acetonitrile/isopropanol (1:1), centrifuged, and transferred to vials for UHPLC-MS analysis as described previously.[19] An appropriate test mixture of standard compounds was analyzed before and after the entire set of randomized sample injections in order to examine the retention time stability, mass accuracy, and sensitivity of the system. Metabolomics data were preprocessed using the TargetLynx application manager for MassLynx 4.1 (Waters Corp, Milford, MA). The $[M + NH_4]^+$ ion adduct of tripalmitin was followed, with a $m/z = 824.771$.

Serum protein biomarkers were measured as follows: PRO-C3 by enzyme-linked immunosorbent assay at Nordic Bioscience (Herlev, Denmark), plasma N-terminal propeptide of type III procollagen (PIIINP) and tissue inhibitor of metalloproteinase 1 (TIMP1) by enzyme-linked immunosorbent assay at Siemens (Berkeley, CA), fibroblast growth factor-21 (FGF-21) by MesoScale Discovery at Precision for Medicine, cytokeratin-18 (CK18) proteolytic fragment CK18 (M30) and intact CK18 (M65) by monoclonal antibody enzyme-linked immunosorbent assay at Cerba.

Outcomes

Safety of the drug for all randomized subjects who received at least 1 dose was the primary safety end point. The primary efficacy end point was the relative change in liver fat between baseline and after 12 weeks of treatment measured by MRI-PDFF as described previously.[20] Patients were considered eligible for efficacy analysis per protocol if they had at least 8 weeks of continuous dosing. Secondary outcomes included MRI-PDFF responder criteria assessment as defined by a relative reduction in MRI-PDFF of $\geq$30% given its association with histologic response.[21–23]

Statistical Analyses

Power calculations used the 2-sided Wilcoxon rank sum test to test pairwise treatment differences of the primary efficacy end point for each dose group with placebo at the 0.05 type I error level. If the standard deviation of the primary efficacy end point is 30 and both pairwise treatment differences are 24, then 30 evaluable patients per treatment group provides at least 80% overall power to detect both pairwise treatment differences.

A fixed-sequency approach was taken for the primary end point (relative reduction in MRI-PDFF), whereby if significant at .05 in the 50 mg:pooled placebo comparison, then the analysis was done for 25 mg:pooled placebo. If both of these pairwise comparisons for the primary end point were significant at .05, then this testing strategy was repeated for the key secondary efficacy end point (percent of subjects with at least a 30% reduction in MRI-PDFF). This approach maintains a study-wide α level at .05 for these comparisons. Each TVB-2640 dose group was compared to the pooled placebo group using an F test from an analysis of covariance model with fixed effects for the stratification factor (diabetes presence/absence) and treatment group (ie, TVB-2640 dose groups and pooled placebo) and with the baseline MRI-PDFF value as a covariate.

Sagimet Biosciences funded this study and employees and consultants of the company participated in the study design, patient safety, data analyses, and writing of the manuscript. All authors had access to the study data and reviewed and approved the final manuscript.

Results

A total of 416 subjects were screened at 10 clinical sites with NASH expertise in the United States and 99 were randomized. Forty-nine patients were randomly assigned to receive either placebo or 25 mg TVB-2640 in a 1:2 ratio.

After an interim assessment of safety by the independent Safety Review Committee, the trial continued as planned by enrolling an additional 50 patients who were randomly assigned to receive either placebo or 50 mg TVB-2640 in a 1:2 ratio (Supplementary Figure 1). All 99 patients were included in the safety assessment; the 85 patients who had an end-of-treatment MRI-PDFF treatment were included in the efficacy and biomarker analyses. Two patients discontinued the study early due to a treatment-emergent adverse event (TEAE) and 5 patients had an end of treatment MRI-PDFF later than planned between 12 and 16 weeks of treatment as a result of COVID-19 visit restrictions and were not included in the primary efficacy analysis.

Baseline demographics and disease characteristics were generally consistent among the groups as well as between the 2 placebo cohorts enrolled during the 2 separate time periods. The median age was 55 years, 46% of patients were female, and most were White, with 72% identifying as Hispanic or Latino. As expected for a NASH population, the median liver fat content was 15.6%, the majority of patients had T2D and the median body mass index was 32.6 kg/m^2 (Table 1). Other disease characteristics were consistent with NASH, including the median magnetic resonance elastography liver stiffness of 3.0 kPa (consistent with the presence of fibrosis) and 12 patients had a pre-existing liver biopsy with fibrosis stages ranging from F1 to F3.

Drug levels in the plasma were consistent with prior studies of TVB-2640 in humans; the half-life in the plasma was approximately 10–12 hours, C_{max} occurred approximately at 4–5 hours and steady-state was evident by day 8 with an approximately 1.3- to 1.6-fold higher exposure compared to day 1, and relatively low variability between patients (Figure 1A). The overall exposure of drug in the 25-mg cohort (area under the curve$_{0-24}$ 6580 ng/h * mL) was increased by 2.6-fold in the 50-mg cohort (area under the curve$_{0-24}$ 16,800 ng/h * mL), consistent with a linear dose proportionality, observed in prior studies with doses ranging from 25 mg to 200 mg in humans (data on file).

Table 1. Patient Demographic Characteristics

Characteristic	Placebo (n = 31)	25 mg (n = 33)	50 mg (n = 35)
Male, n (%)	14 (45.2)	18 (54.5)	22 (62.9)
T2D, n (%)	17 (54.8)	25 (75.8)	13 (37.1)
Ethnicity, *Hispanic*, n (%)	25 (80.6)	22 (66.7)	24 (68.6)
Age, *y*, median (Q1, Q3)	52 (46, 58)	58 (53, 62)	55 (44, 62)
Weight, *kg*, median (Q1, Q3)	83.7 (74.0, 96.8)	95.4 (84.9, 105.6)	92.0 (83.0, 101.0)
Body mass index, *kg/m^2*, median (Q1, Q3)	31.2 (29.3, 35.1)	34.0 (29.7, 38.1)	32.8 (29.6, 35.2)
ALT, *U/L*, median (Q1, Q3)	25 (16, 46)	28 (23, 36)	29 (24, 43)
AST, *U/L*, median (Q1, Q3)	21 (15, 30)	21 (17, 26)	23 (20, 30)
Alkaline phosphatase, *U/L*, median (Q1, Q3)	82 (72, 98)	76 (62, 92)	74 (58,103)
GGT, *U/L*, median (Q1, Q3)	33 (22, 58)	32 (22, 40)	39 (25, 49)
Glucose (fasting), *mg/dL*, median (Q1, Q3)	108 (86, 167)	152 (103, 187)	98 (80, 124)
HbA1c, %, median (Q1, Q3)	6.4 (5.9, 8.6)	7.1 (6.2, 8.3)	5.8 (5.5, 6.4)
Insulin (fasting) *μU/mL*, median (Q1, Q3)	17 (15, 24)	23 (13, 37)	22 (14, 32)
HOMA-IR, median (Q1, Q3)	5.7 (3.7, 6.7)	8.4 (4.0, 14.1)	5.0 (3.7, 7.8)
Apolipoprotein B, *mg/dL*, median (Q1, Q3)	100 (84, 126)	109 (90, 117)	104 (89, 124)
Total-chol, *mg/dL*, median (Q1, Q3)	192 (162, 229)	194 (161, 203)	189 (167, 225)
LDL-chol, *mg/dL*, median (Q1, Q3)	116 (98, 139)	127 (104, 136)	114 (94, 153)
HDL-chol, *mg/dL*, median (Q1, Q3)	43 (39, 53)	40 (36, 54)	44 (37, 51)
TG, *mg/dL*, median (Q1, Q3)	157 (123, 248)	159 (113, 218)	163 (124, 262)
CK18 (M30), *U/L*, median (Q1, Q3)	224 (174, 401)	301 (209, 506)	257 (159, 476)
MRI-PDFF, %, median (Q1, Q3)	15.3 (11.8, 22.2)	14.3 (10.4, 22.3)	15.8 (12.3, 19.6)
MRE, *kPa*, median (Q1, Q3)	3.0 (2.7, 3.4)	2.9 (2.7, 3.2)	3.0 (2.8, 3.2)
PRO-C3, *ng/mL*, median (Q1, Q3)	15.2 (10.85, 18.60)	17.3 (14.4, 19.80)	17.0 (12.7, 25.3)

AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; HbA1c, hemoglobin A1c; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; MRE, magnetic resonance elastography; Q1, first quartile; Q3, third quartile.

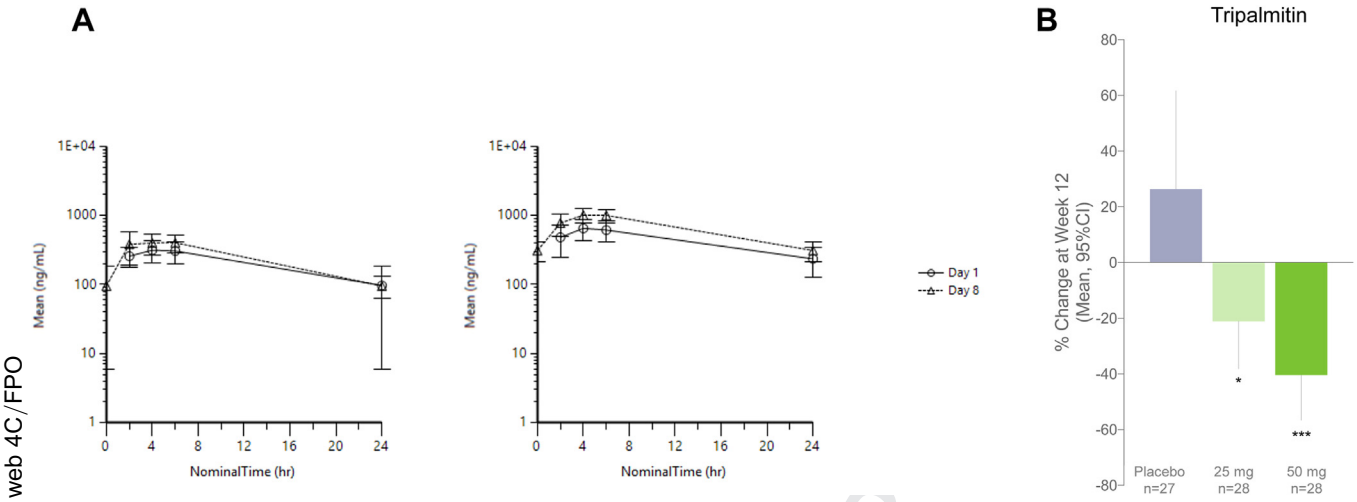

Figure 1. Pharmacokinetic and pharmacodynamic assessments. (*A*) Plasma pharmacokinetic profile of TVB-2640 in 25-mg and 50-mg cohorts. Plasma levels of TVB-2640 were measured on day 1 (2, 4, 6, and 24 hours post dose) and day 8 (pre-dose, 2, 4, and 6 hours post dose). On day 8, the predose value is also graphed at 24 hours. Mean/standard deviation of n = 12 (25 mg) and 16 (50 mg). (*B*) Tripalmitin, pharmacodynamic marker of FASN inhibition. Tripalmitin levels in plasma were quantitated by UHPLC-MS. Results are presented as pairwise % change at week 12 compared to baseline. *$P < .05$; ***$P < .001$ vs placebo from an analysis of covariance model with fixed effects for the stratification factor (diabetes and treatment group), with baseline value as a covariate.

FASN inhibition was assessed by measurement of serum tripalmitin, a triglyceride (TG) in which all 3 acyl chains are palmitate and its serum level provides an estimate of DNL.[24] Tripalmitin increased by 26% in the placebo group and was reduced by 21% in the 25-mg cohort and by 40% in the 50-mg cohort ($P < .01$ and $P < .0001$, respectively) after 12 weeks of treatment (Figure 1*B*). Decreased tripalmitin was evident by week 4 of dosing at 50 mg with a median decrease of 21% ($P < .05$) from baseline, the earliest post-baseline timepoint tested (not tested for 25 mg). The reduction in this pharmacodynamic marker demonstrates significant inhibition of FASN and hepatic DNL by TVB-2640.

Safety data were collected from a total of 99 patients, of whom 68 were treated with TVB-2640. Overall, 62 patients (63%) experienced at least 1 TEAE, all of which were assessed by the investigator as grade 1/mild except for 1 grade 2 urinary tract infection and 1 increased appetite at 25 mg, and 1 shortness of breath at 50 mg; all 3 resolved without dose adjustment. No on-treatment serious adverse events occurred in any dose group. Overall, the most common TEAEs, regardless of drug-relatedness, among TVB-2640–treated patients were headache (6 patients [9%]), peripheral edema, rash, and upper respiratory tract infection (4 patients [6%]), and bronchitis, diarrhea, nausea, and urinary tract infection (3 patients [4%]) and hypertriglyceridemia (noted as unrelated to treatment; 2 patients [5.7%]) (Table 2). Two patients (3%) discontinued TVB-2640 due to a TEAE, 1 due to mild eye allergy on day 2 of study and the other due to mild conjunctivitis; both at the 25-mg dose; no discontinuations for a TEAE occurred in the 50-mg dose cohort (Table 2).

The primary efficacy end point was change in liver fat by MRI-PDFF imaging at 12 weeks.[22,23] TVB-2640 treatment resulted in significant relative and absolute reductions of liver fat compared to placebo in a dose-dependent manner. In the placebo group, relative liver fat increased on average by 4.5% ± 35.9% relative to baseline during the 12-week dosing period. In contrast, there was a relative reduction of liver fat of 9.6% ± 29.1% in the 25-mg cohort ($P = .053$) and 28.1% ± 28.0% ($P = .001$) in the 50-mg cohort (Figure 2*A*). Lean absolute liver fat reduction was 0.3% in the placebo arm compared to 1.8% (P = NS) in the 25-mg arm and 5.1% in the 50 mg arm ($P = .001$) (Figure 2*B*). The overall trends were not significantly impacted by T2D status, magnetic resonance elastography liver stiffness, or liver fat values at baseline. In addition, body weight changes were negligible in each of the 3 cohorts and there was no observed correlation between body weight change and liver fat change. To provide further evidence that the reduction of liver fat was due to the pharmacological activity of TVB-2640, MRI-PDFF was assessed at week 16, four weeks after treatment was stopped. Ninety-three percent of the patients had a third MRI-PDFF measured at week 16. Liver fat in placebo-treated patients was similar between week 16 and week 12 (4.6% and 4.5%, respectively, compared to baseline). In contrast liver fat had begun to increase in both the 25-mg and 50-mg TVB-2640 cohorts (–7.0% and –13.1%, respectively, compared to baseline; not significant compared to placebo).

A dose-dependent increase in response rate was observed among the 3 cohorts and the majority of patients treated with 50 mg TVB-2640 achieved an MRI-PDFF response. Eleven percent (n = 3 of 27) of placebo patients had a ≥30% relative reduction in liver fat content vs 23% (n = 7 of 30) of patients treated with 25 mg TVB-2640 (P = NS) and 61% (17 of 28) of patients treated with 50 mg ($P < .001$) (Figure 2*C*). Furthermore, only 1 patient in the placebo group (3.7%) had a relative reduction of ≥50% liver fat, compared to 4 (13.3%) and 6 (21.4%) in the 25-mg and 50-mg TVB-2640–treated cohorts, respectively (Figure 2*D*).

Table 2. Safety Profile of TVB-2640 in Patients With Nonalcoholic Steatohepatitis

Variable	Placebo (n = 31)	25 mg TVB-2640 (n = 33)	50 mg TVB-2640 (n = 35)
Most common TEAE reported for ≥5% of patients in any cohort (regardless of attribution to drug or not), n (%)			
Headache	2 (6.3)	4 (12.1)	2 (5.7)
Peripheral edema/swelling	0	3 (9.1)	1 (2.9)
Rash	1 (3.1)	3 (9.1)	1 (2.9)
Upper respiratory infection	1 (3.1)	2 (6.1)	2 (5.7)
Bronchitis	0	3 (9.1)	0
Diarrhea	1 (3.1)	3 (9.1)	0
Hypertriglyceridemia	2 (6.3) 1	0	2 (5.7)[a]
Nausea	0	3 (9.1)	0
Urinary tract infection	0	2 (6.1)	1 (2.9)
Summary of TEAEs			
Any			
Grade 1	11 (35)	18 (54.5)	11 (31.4)
Grade 2	8 (25.8)	7 (21.2)	7 (20.0)
Leading to drug withdrawal	0	2 (6.1)	0
Serious adverse event	0	0	0
Death	0	0	0
Drug-related			
Grade 1	3 (9.7)	10 (30.3)	9 (25.7)
Grade 2	1 (3.2)	2 (6.1)[b]	1 (2.9)[c]

[a]These hypertriglyceridemia events unrelated to drug.
[b]25 mg: urinary tract infection and increased appetite, both resolved without dose adjustment.
[c]50 mg: shortness of breath, resolved without dose adjustment.

Markers of liver injury improved on treatment with TVB-2640. Consistent with the relative increase of liver fat in the placebo cohort, plasma ALT levels increased by 3.4 U/L (7.4%) over 12 weeks (Figure 3*A*). In contrast, ALT levels decreased at both doses of TVB-2640. Levels decreased by 3.6 U/L (3%; P = NS) and 12.3 U/L (22.3%) ($P < .005$) from baseline in the 25-mg and 50-mg cohorts, respectively, with a mean difference of 13.6 U/L in the 50-mg cohort compared to placebo ($P < .005$). The decrease in ALT was both dose-dependent and time-dependent, evident at week 2, 4, and 8 at 50 mg with highest decrease at week 12 (Figure 3*B*). Approximately one-third of the patients in each cohort had abnormal ALT levels at baseline. In this subgroup, 33% of placebo patients normalized ALT post-treatment compared to 60% of the patients treated with 50 mg of TVB-2640.[25] Among patients with elevated ALT at baseline, 33% of those in the placebo cohort achieved a ≥17 U/L reduction vs 50% of patients treated with 50 mg TVB-2640. Liver fat reduction was generally associated with ALT decreases in TVB-2640–treated patients but not placebo-treated patients.

Similar to ALT, serum aspartate aminotransferase levels increased in the placebo group over 12 weeks but decreased in a dose-dependent manner in TVB-2640–treated patients

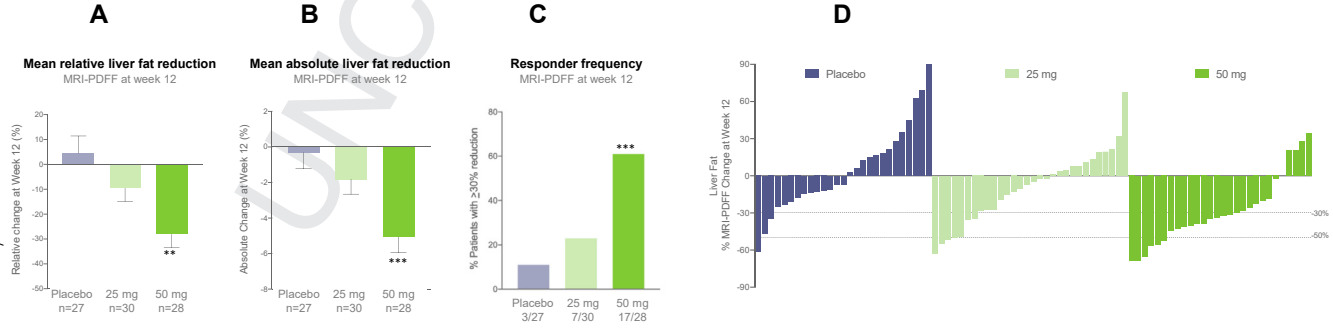

Figure 2. Changes in liver fat content measured by MRI-PDFF. (*A*) Relative change (percent change) of liver fat at week 12 relative to baseline (mean/standard error of mean [SEM]). (*B*) Absolute change of liver fat at week 12 relative to baseline (mean/SEM). (*C*) Percent of patients with a ≥30% relative decrease of liver fat. (*D*) Relative liver fat change at week 12 relative to baseline for each patient per treatment group. *Dashed lines* represent the 30% relative reduction and 50% relative reduction levels. $^{**}P < .005$; $^{***}P < .001$ vs placebo from an analysis of covariance model with fixed effects for the stratification factor (diabetes and treatment group), with baseline value as a covariate.

($P < .05$) (Figure 3*A*). Small and/or non–dose-dependent changes were noted in γ-glutamyl transpeptidase (Figure 3*A*) and alkaline phosphatase (Figure 5).

Another important indicator of liver damage in NASH patients is the elevation of serum proteolytic fragments of CK18, the major intermediate filament protein of hepatocytes. CK18 (M30) increased by 54.7% in the placebo group over 12 weeks of this study, consistent with continued liver damage; in contrast, there was a lesser increase in the 25-mg cohort (9.3%; $P = .04$) and an 11.7% decrease in patients treated with 50 mg TVB-2640 ($P = .006$) (Figure 3*C*). Similar changes were observed in intact CK18 (M65). Reduction of these serum markers suggests improved hepatocyte viability in TVB-2640–treated patients with NASH.

Hyperlipidemia was common in these patients. Total cholesterol (Tot-chol), high-density lipoprotein cholesterol (HDL-chol), and low-density lipoprotein cholesterol (LDL-chol) levels increased by 4.7%, 5.2% and 9%, respectively, in the placebo cohort (Figure 3*D*). In contrast, patients treated with 50 mg TVB-2640 exhibited decreases in all cholesterol fractions with decreases of 5.1% ($P = .05$) for Tot-chol, 11% ($P = .01$) for LDL-chol, and 4.4% ($P = .004$) for HDL-chol. Baseline ratios of Tot-chol/HDL were elevated at 4.4–4.6 and there were no changes to these ratios after treatment (Figure 5), suggesting that the HDL-chol decrease was a consequence of decreased Tot-chol levels. ApoB also increased in the placebo group, consistent with elevation of LDL and very low-density lipoprotein particles in the serum. Consistent with reductions of LDL-chol in the TVB-2640 cohorts, ApoB levels were lower in drug-treated patients relative to placebo, although the change was not significant. TG levels varied widely among patients and with repeated measures in the same patients with no significant changes between placebo and drug-treated groups. No laboratory AEs of drug-related hypertriglyceridemia were found.

FGF-21 and adiponectin are important regulators of insulin sensitivity. Elevated levels of FGF-21 in obese patients may be indicative of a protective response to preserve insulin sensitivity. FGF-21 levels increased by 82% and adiponectin by 17.4% in the 50-mg arm ($P < .005$ and $P < .05$,

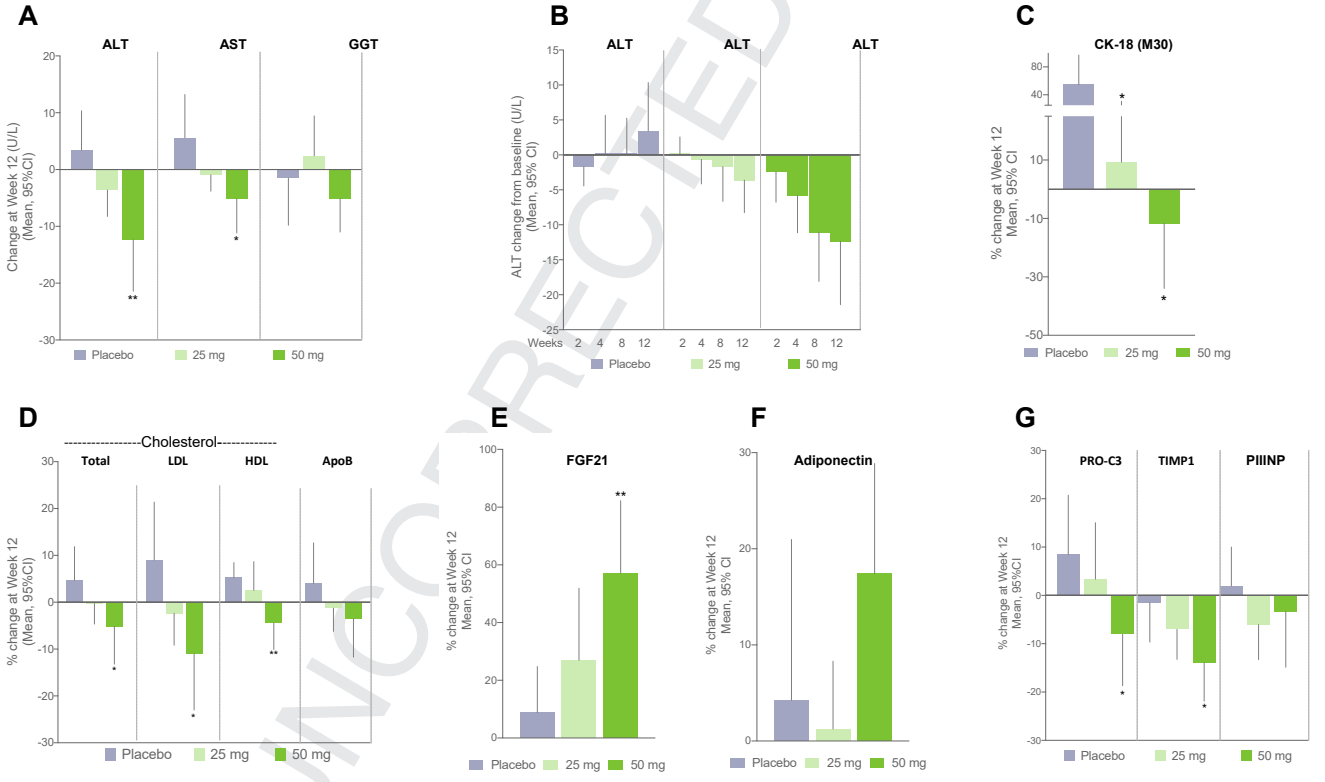

Figure 3. Changes in plasma biomarkers. (*A*) Absolute change in ALT, aspartate aminotransferase, and γ-glutamyl transpeptidase (U/L) at week 12 compared to baseline, n of 27, 30, 27 for placebo, 25 mg, 50 mg respectively. (*B*) ALT absolute change from baseline (U/L) at 2, 4, 8, and 12 weeks of treatment. $^{*}P < .05$, $^{**}P < .005$ vs placebo with fixed effects for the stratification factor (diabetes and treatment group), with baseline value as a covariate. (*C*) Percent change in CK18 (M30) (U/L) at week 12 compared to baseline, n of 25, 30, 21 for placebo, 25 mg, 50 mg respectively. (*D*) Percent change in cholesterol fractions (mg/dL) and ApoB (mg/dL) at week 12 compared to baseline, n of 28, 29, 27 for placebo, 25 mg, 50 mg respectively, $^{*}P < .05$; $^{**}P < .005$ vs placebo with fixed effects for the stratification factor (diabetes and treatment group), with baseline value as a covariate. (*E*) Percent change in FGF-21 levels (pg/mL) at week 12 compared to baseline, n of 27, 24, 27 for placebo, 25 mg, 50 mg respectively. (*F*) Percent change in adiponectin levels (μg/mL) at week 12 compared to baseline. n of 27, 30, 26 for placebo, 25 mg, 50 mg respectively. $^{**}P < .005$ by Mann Whitney U test vs placebo. (*G*) Percent change in PRO-C3 (ng/mL), TIMP-1 (ng/mL), and PIIINP (ng/mL) between baseline and week 12. n of 27, 28, 28 (ProC3) and 27, 27, 26 (TIMP1 and PIIINP) for placebo, 25 mg, 50 mg respectively. $^{*}P < .05$ by Mann Whitney U test vs placebo.

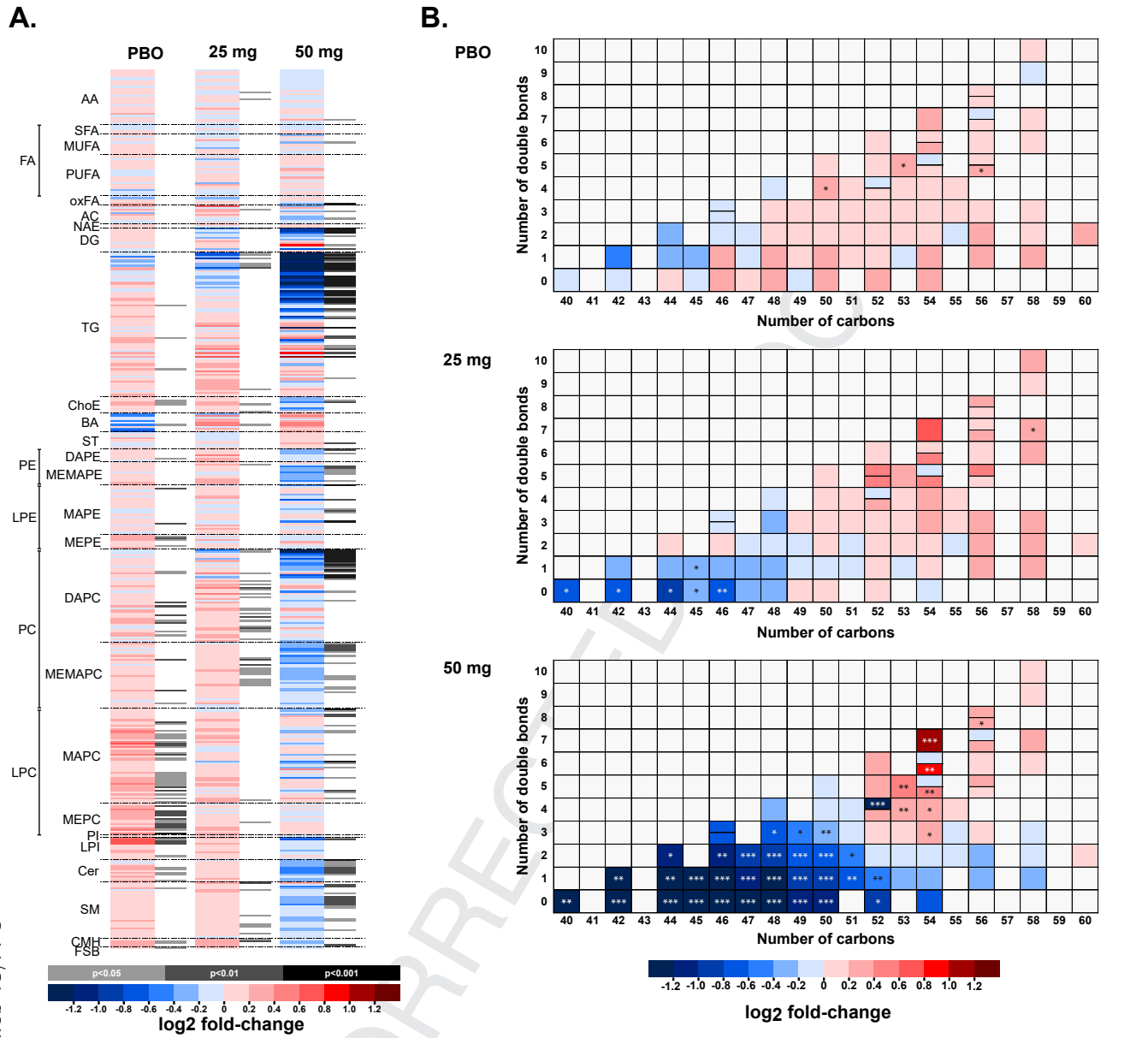

Figure 4. Lipidomic profiling of patient serum. (*A*) Lipidomic profiling of 400 species performed by UHPLC-MS. *Heatmap* of binary paired comparisons of week 12 to baseline for placebo (n = 25), 25 mg TVB-2640 (n = 27), and 50 mg TVB-2640 (n = 28) as $\log_2$ pairwise fold change. Lipids are grouped by class as indicated for a total of approximately 400 species. *Red* indicates increase and *blue* indicates decrease. Statistical significance is indicated in *gray scale* for each species. (*B*) *Heatmap* displaying the changes (week 12 compared to baseline) in TG species with respect to number of carbons and double bonds. *Color code* represents the transformed ratios between means of the groups ($\log_2$ [fold-change]). Student *t* test *P* values (or Welch's *t* test where unequal variances were found): $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$. AA, amino acids; AC, acyl carnitines; ArAA, aromatic amino acids; BA, bile acid; BCAA, branched chain amino acids; Cer, ceramides; ChoE, cholesteryl ester; CMH, monohexosylceramides (Cerebrosides); DAPE, diacylglycerophosphatidylethanolamines (DAPE); DAPC, diacylglycerophosphatidylcholines; DG, diglycerides; FSB, free sphingoid base; LPC, lysophosphatidylcholines; LPE, lysophosphatidylethanolamines; LPI, lysophosphatidylinositols; MAPC, monoacylclycerophosphocholines; MAPE, monoacylglycerophosphoethanolamine; MAPI, monoacylglycerophosphoinositol; MEMAPC, 1-ether, 2-acylglycerophosphocholines; MEMAPE, 1-ether, 2-acylglycerophosphoethanolamine; MEPC, monoetherglycerophosphocholines; MEPE, 1-monoetherglycerophosphoethanolamine; MUFA, monounsaturated fatty acid; NAE, N-acyl ethanolamines; NEFA, nonesterified fatty acid; PC, phosphatidylcholines; PE, phosphatidylethanolamine; PG, phosphatidylglycerols; PI, phosphatidylinositols; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acid; SM, sphingomyelin; TG, triglycerides; UFA, unsaturated fatty acid.

respectively, vs placebo) (Figure 3*E* and *F*). There were no significant changes in fasting glucose or insulin levels at week 12.

Treatment of patients with TVB-2640 had a significant impact on several serum markers of fibrosis in this 12-week study. TIMP-1, PRO-C3, and PIIINP all decreased in a dose-

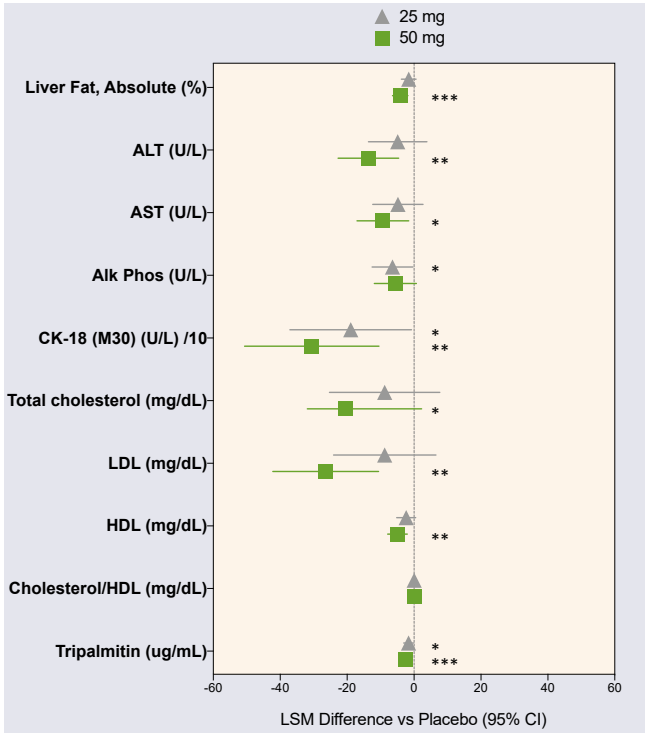

Figure 5. Summary of effect of TVB-2640 on tripalmitin, liver injury markers and cholesterol profiles. Difference in least square (LS) means (absolute) is shown for 25 mg TVB-2640 and 50 mg TVB-2640 vs placebo. LS mean estimates are based on pairwise comparisons with fixed effects for the stratification factor (diabetes and treatment group), and with baseline value as a covariate. *$P < .05$; **$P < .005$; ***$P < .001$ vs placebo.

dependent manner in patients treated with TVB-2640. The PRO-C3 epitope is specific for cleaved procollagen 3 and its presence in the blood is a marker of active fibrogenesis in the liver. PRO-C3 levels increased in the placebo group by 8.5% and decreased in the TVB-2640 50-mg-treated cohort by 8.1% ($P = .042$); TIMP-1, another marker of stellate cell activation, was reduced significantly by 18% ($P < .05$) from baseline. An assay for PIIINP (detects both cleaved and uncleaved procollagen 3) also exhibited a dose-dependent decrease treatment, although the change was not significant (Figure 3*G*).

The significant reductions of liver fat, serum tripalmitin, and cholesterol (summarized in Figure 5) warranted a more detailed evaluation of global lipid changes in the serum of these patients, particularly those related to lipotoxic species associated with pathogenesis of liver and metabolic disease. Blood samples collected at baseline, 4 weeks, and 12 weeks of treatment were evaluated for more than 450 different metabolites by UHPLC-MS, especially free fatty acids and their derivatives, bile acids, and sterols/steroids. As evidenced by the heatmap, with the exception of increases in monoetherglycerophosphocholine and monoacylglycerophosphocholine species there were no other classes that significantly changed in the placebo cohort at week 12, indicating that time was not a significant factor driving changes in metabolite profiles (Figure 4*A*). As expected, free fatty acids in plasma, including palmitate, showed no or minimal differences among the placebo and TVB-2640 groups after 12 weeks of treatment. In addition, a subset of acyl carnitines was measured (acyl chains of 8:0, 10:0, 12:0, 14:2n-x, or 16:0) and there were no significant changes from baseline. In contrast, TGs enriched for palmitate-containing species (shorter carbon chains and higher degree of saturation), including but not limited to tripalmitin, were significantly reduced in patients treated with 25 mg or 50 mg TVB-2640 in a dose-dependent manner (Figure 4*B*). In contrast some TGs consistent with long chain polyunsaturated acyl chains increased in these subjects. Consistent with palmitate as a major substrate for ceramide and DAG synthesis, TVB-2640 reduced certain DAGs and bile acids, and caused broad decreases across the ceramides and sphingomyelins, especially those containing saturated acyl chains with 16–18 carbons. Most of these changes were observed by 4 weeks of treatment in patients treated with 50 mg TVB-2640. These favorable decreases are consistent with DNL pathway inhibition, further confirming the effects of TVB-2640 on the generation of lipids responsible for lipotoxicity, the hallmark driver of NASH.

Discussion

In this randomized, placebo-controlled study, TVB-2640 was well tolerated and produced a significant dose-dependent reduction of liver fat content after 12 weeks of treatment. A 50-mg daily oral dose of TVB-2640 for 12 weeks reduced liver fat on average by 28.1%, and 61% of subjects achieved an MRI-PDFF response of ≥30% relative reduction of liver fat. This drug produced a multipronged effect on lipids in NASH patients: inhibition of DNL, reduction of hepatic fat (MRI-PDFF), and levels of lipotoxic molecules, including DAGs and ceramides produced from excess palmitate. In addition, the mechanism of action of TVB-2640 is expected to reduce liver fibrosis by virtue of the critical role of FASN in activation of fibrogenesis in hepatic stellate cells.[16] TVB-2640 treatment of NASH patients resulted in a significant reduction of serum biomarkers of fibrosis, PRO-C3 and TIMP-1, despite the short duration of treatment. The placebo population in this study showed increases in liver fat, liver enzymes, CK18, and cholesterol levels over 12 weeks. In contrast, TVB-2640 elicited a rapid impact on DNL, hepatic fat, blood levels of pro-inflammatory/pro-fibrotic lipotoxic metabolites and markers of fibrogenesis, which aligns with the mechanism of action of this drug across multiple pathologic pathways, suggesting its potential as a treatment for patients with NASH (Figure 5).

In other studies, NASH patients who achieved an MRI-PDFF response (relative reduction of ≥30% liver fat), after 12–24 weeks of treatment have been shown later to have improved liver histology on repeat liver biopsy, and this response has emerged as a strong and important indicator of NASH histologic improvement.[21,23,26] Patients with an MRI-PDFF response have a 7-fold increase in the improvement of NAFLD Activity Score by 2 or more points, a 5-fold increase in the rate of NASH resolution and improved fibrosis compared to those who do not achieve a

response.[21,27] These rates of histologic improvement are likely even higher in subjects who achieve a ≥50% reduction of liver fat by MRI-PDFF.[28] In this trial, 61% of NASH patients treated with TVB-2640 for only 12 weeks achieved a ≥30% reduction of liver fat by MRI-PDFF and 21% achieved a ≥50% reduction of liver fat. Future studies will determine whether a longer duration of treatment will result in an even greater response. Because there was a small number of patients in the 50-mg cohort who did not reduce liver fat, there may be a subset of NASH patients in whom DNL is not a major driver of disease and they might not respond to drugs that reduce DNL.

In addition to the reduction of liver fat, other hallmarks of liver health improved after treatment with TVB-2640. Both ALT and CK18 levels were reduced by TVB-2640 treatment vs placebo but, in contrast, these markers increased in placebo patients. Importantly, half of the patients with baseline ALT at or above the upper level of normal treated with 50 mg of TVB-2640 achieved a ≥17 U/L reduction of ALT, a threshold that is associated with improvement of liver fibrosis on histological examination.[25]

NASH is a complex disease and patients often have cardiovascular complications resulting from the metabolic syndrome, T2D, and dyslipidemia. TVB-2640 treatment was associated with metabolic improvements. Inhibition of ACC reduces DNL while increasing plasma levels of TGs[18,29]; in contrast, FASN inhibition with TVB-2640 reduced DNL without the negative consequence of TG elevation. A significant decrease in TGs with shorter, more saturated acyl chains may have been offset by a similar increase in TGs with longer, polyunsaturated acyl chains resulting in no net change. In addition, LDL-chol was reduced by up to 18.7% (95% confidence interval, −33.1% to −4.4%; $P = .01$) compared to placebo, which suggests that long-term treatment can improve the cardiovascular risk. FGF-21 and adiponectin levels were significantly increased, indicative of a protective response to restore insulin sensitivity especially in obese subjects.[30,31] FGF-21 and adiponectin rely on each other for maximal activity and restoration of insulin sensitivity. Although there were no significant changes in insulin levels or fasting blood glucose in the current study, these measures will be reassessed in future studies with a longer treatment duration.

Because the extent of fibrosis is a leading prognostic indicator of progression to cirrhosis and its complications, it is imperative that therapies either regress fibrosis or blunt the progression of fibrosis. TVB-2640 produced a sharp decline of the serum fibrosis markers PRO-C3, TIMP-1, and PIIINP in only 12 weeks, indicating that the drug might have a direct impact on stellate cells and fibrogenesis in these patients. The impact of TVB-2640 treatment on fibrosis may be 2-fold. One path is indirect; mediated by reduction of lipotoxic liver injury that drives fibrosis-inducing damage. The other is a more direct effect on inflammatory stimuli and stellate cells in the liver, as these cell types rely on DNL for activation. Inhibition of FASN and DNL in human hepatic stellate cells studied ex vivo reduces procollagen expression, profibrotic gene expression and prevents activation of these cells to myofibroblasts.[16] These indirect and direct actions have the potential to improve fibrosis while on therapy, which will be further evaluated in future trials with histological end points.

A major emerging topic in the pathogenesis of NASH is the role of lipotoxic species in causing and exacerbating liver damage. Ceramides, for example, are elevated in patients with NASH and bariatric surgery is associated with a decline in these lipotoxic metabolites. Palmitate itself has inherent inflammatory properties either directly by stimulating receptors, such as TLR4, or indirectly as a substrate for production of other lipotoxins. DAGs and ceramides also play a significant role in insulin resistance. Certain DAGs activate protein kinase C isoforms in the muscle and liver, and ceramides inactivate Akt/PI3K signaling in response to insulin: both these mechanisms contribute to insulin resistance.[12,13] This early-phase study of a FASN inhibitor shows beneficial effects on potential lipotoxic mediators of liver injury in NASH as reflected by lower ceramide levels in blood.

Prior studies have shown that relatively high doses of TVB-2640 can reduce serum tripalmitin levels by 10%–15% after 1–2 weeks of treatment.[32] The current study demonstrated that 12 weeks of dosing with TVB-2640 lowered tripalmitin levels in a dose-dependent fashion as much 40%. At a minimum tripalmitin may serve as a useful pharmacodynamic marker of FASN inhibition. Further studies correlating a direct measurement of DNL to changes in tripalmitin and liver fat in NASH patients may help define the utility of this marker.

The good safety profile of TVB-2640 in these patients was consistent with previous studies; there were no drug-related organ, metabolic or skin toxicities reported. TVB-2640 has been given to oncology patients at doses of 150 mg or higher per day for months without any notable impacts on liver tissue or other major internal organs. At these several-fold higher doses used in cancer patients, lipogenesis is inhibited in the skin and can lead to dry skin, dry eye, and hair thinning, which can be treated with eye drops or lotions, and are reversible.[33] At these high doses, 3 times the 50-mg dose used in this study, and left untreated, these conditions can lead to palmar-plantar erythrodysesthesia, iritis/uveitis, or alopecia, respectively, in some patients.

The limitations of the current study include the relatively small sample size and short duration of dosing can confound the assessment of biomarkers of fibrosis in patients' plasma; additional studies will be needed to directly assess the impact of TVB-2640 on liver histology. T The worsening of multiple parameters in the placebo group is uncommon in these studies and there were limited assessments of potential confounding effects of diet, exercise and/or alcohol use. In addition the single-blind design cannot eliminate the possibility that placebo and TVB-2640–treated patients were differentially advised on behaviors by unblinded investigators.

TVB-2640 potently reduced liver fat in NASH patients with a majority achieving an MRI-PDFF response within 12 weeks of treatment. This effect combined with biomarkers showing reduction in liver injury, improvement in metabolic function, reduction of pro-inflammatory lipotoxins, evidence

of reduced fibrogenesis, and a favorable effect on serum lipids combine to provide a compelling foundation for larger randomized clinical trials with histologic end points.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://doi.org/10.1053/j.gastro.2021.07.025.

References

1. Simon TG, Roelstraete B, Khalili H, et al. Mortality in biopsy-confirmed nonalcoholic fatty liver disease: results from a nationwide cohort. Gut 2020. gutjnl-2020-322786.
2. Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 2017; 15:11.
3. Sheka AC, Adeyi O, Thompson J, et al. Nonalcoholic steatohepatitis. JAMA 2020;323:1175–1183.
4. Friedman SL, Neuschwander-Tetri BA, Rinella M, et al. Mechanisms of NAFLD development and therapeutic strategies. Nat Med 2018;24:908–922.
5. Kusminski CM, Scherer PE. Lowering ceramides to overcome diabetes. Science 2019;365:319–320.
6. Marra F, Svegliati-Baroni G. Lipotoxicity and the gut-liver axis in NASH pathogenesis. J Hepatol 2018;68:280–295.
7. Smith GI, Shankaran M, Yoshino M, et al. Insulin resistance drives hepatic de novo lipogenesis in nonalcoholic fatty liver disease. J Clin Invest 2019;130:1453–1460.
8. Ogawa Y, Imajo K, Honda Y, et al. Palmitate-induced lipotoxicity is crucial for the pathogenesis of nonalcoholic fatty liver disease in cooperation with gut-derived endotoxin. Sci Rep-uk 2018;8:11365.
9. Dong Z, Zhuang Q, Ning M, et al. Palmitic acid stimulates NLRP3 inflammasome activation through TLR4-NF-κB signal pathway in hepatic stellate cells. Ann Transl Medicine 2020;8:168.
10. Snodgrass RG, Huang S, Namgaladze D, et al. Docosahexaenoic acid and palmitic acid reciprocally modulate monocyte activation in part through endoplasmic reticulum stress. J Nutritional Biochem 2016;32:39–45.
11. Galadari S, Rahman A, Pallichankandy S, et al. Role of ceramide in diabetes mellitus: evidence and mechanisms. Lipids Health Dis 2013;12:98.
12. Pagadala M, Kasumov T, McCullough AJ, et al. Role of ceramides in nonalcoholic fatty liver disease. Trends Endocrinol Metabolism 2012;23:365–371.
13. Erion DM, Shulman GI. Diacylglycerol-mediated insulin resistance. Nat Med 2010;16:400–402.
14. Chakravarthy MV, Pan Z, Zhu Y, et al. "New" hepatic fat activates PPARα to maintain glucose, lipid, and cholesterol homeostasis. Cell Metab 2005;1:309–322.
15. Wei X, Song H, Yin L, et al. Fatty acid synthesis configures the plasma membrane for inflammation in diabetes. Nature 2016;539:294–298.
16. Bates J, Vijayakumar A, Ghoshal S, et al. Acetyl-CoA carboxylase inhibition disrupts metabolic reprogramming during hepatic stellate cell activation. J Hepatol 2020.
17. Syed-Abdul MM, Parks EJ, Gaballah AH, et al. First-in-class fatty acid synthase inhibitor TVB-2640 reduces hepatic de novo lipogenesis in males with metabolic abnormalities. Hepatology 2019;72(1).
18. Loomba R, Kayali Z, Noureddin M, et al. GS-0976 reduces hepatic steatosis and fibrosis markers in patients with nonalcoholic fatty liver disease. Gastroenterology 2018;155:1463–1473.e6.
19. Barr J, Caballería J, Martínez-Arranz I, et al. Obesity-dependent metabolic signatures associated with nonalcoholic fatty liver disease progression. J Proteome Res 2012;11:2521–2532.
20. Dulai PS, Sirlin CB, Loomba R. MRI and MRE for non-invasive quantitative assessment of hepatic steatosis and fibrosis in NAFLD and NASH: clinical trials to clinical practice. J Hepatol 2016;65:1006–1016.
21. Stine JG, Munaganuru N, Barnard A, et al. Change in MRI-PDFF and histologic response in patients with nonalcoholic steatohepatitis: a systematic review and meta-analysis [published online ahead of print August 21, 2020]. Clin Gastroenterol Hepatol doi:10.1016/j.cgh.2020.08.061
22. Loomba R, Neuschwander-Tetri BA, Sanyal A, et al. Multicenter validation of association between decline in MR-PDFF and histologic response in NASH. Hepatology 2020;72:1219–1229.
23. Loomba R. MRI-PDFF treatment response criteria in nonalcoholic steatohepatitis. Hepatology 2021;73:881–883.
24. Aarsland A, Wolfe RR. Hepatic secretion of VLDL fatty acids during stimulated lipogenesis in men. J Lipid Res 1998;39:1280–1286.
25. Loomba R, Sanyal AJ, Kowdley KV, et al. Factors associated with histologic response in adult patients with nonalcoholic steatohepatitis. Gastroenterology 2018; 156:88–95.e5.
26. Jayakumar S, Middleton MS, Lawitz EJ, et al. Longitudinal correlations between MRE, MRI-PDFF, and liver histology in patients with non-alcoholic steatohepatitis: analysis of data from a phase II trial of selonsertib. J Hepatol 2018;70:133–141.
27. Tamaki N, Munaganuru N, Jung J, et al. Clinical utility of 30% relative decline in MRI-PDFF in predicting fibrosis regression in non-alcoholic fatty liver disease. Gut 2021. gutjnl-2021-324264.
28. Loomba R, Bedossa P, Guy C, et al. Magnetic resonance imaging-proton density fat fraction (MRI-PDFF) to predict treatment response on NASH liver biopsy; a secondary analysis of the resmetirom randomized placebo controlled Phase 2 clinical trial. J Hepatol 2020;73.
29. Kim C-W, Addy C, Kusunoki J, et al. Acetyl CoA carboxylase inhibition reduces hepatic steatosis but elevates plasma triglycerides in mice and humans: a bedside to bench investigation. Cell Metab 2017;26:394–406.e6.
30. Holland WL, Adams AC, Brozinick JT, et al. An FGF21-adiponectin-ceramide axis controls energy expenditure and insulin action in mice. Cell Metab 2013;17:790–797.

31. Tillman EJ, Rolph T. FGF21: an emerging therapeutic target for non-alcoholic steatohepatitis and related metabolic diseases. Front Endocrinol 2020;11:601290.
32. Brenner A, Infante J, Patel M, et al. Abstract A54: First-in-human study of the first-in-class fatty acid synthase (FASN) inhibitor, TVB-2640 as monotherapy or in combination–final results of dose escalation. Mol Cancer Ther 2015;14.
33. Falchook G, Infante J, Arkenau H-T, et al. First-in-human study of the safety, pharmacokinetics, and pharmacodynamics of first-in-class fatty acid synthase inhibitor TVB-2640 alone and with a taxane in advanced tumors. Eclinicalmedicine 2021;34:100797.

Received May 7, 2021. Accepted July 13, 2021.

Correspondence
Address correspondence to: Rohit Loomba, MD, MHSc, Nonalcoholic Fatty Liver Disease Research Center, Division of Gastroenterology and Hepatology, Department of Medicine, University of California at San Diego, ACTRI Building, 1W202, 9452 Medical Center Drive, La Jolla, California 92037. e-mail: roloomba@ucsd.edu.

CRediT Authorship Contributions
Rohit Loomba, MD, MHSc (Conceptualization: Lead; Formal analysis: Equal; Methodology: Equal; Supervision: Lead; Writing – review & editing: Equal). Rizwana Mohseni, DO (Formal analysis: Supporting; Writing – review & editing: Supporting; patient care: Equal). K. Jean Lucas, MD (Formal analysis: Supporting; Writing – review & editing: Supporting; patient care: Equal). Julio A. Gutierrez, MD (Formal analysis: Supporting; Writing – review & editing: Supporting; patient care: Equal). Robert G. Perry, MD (Formal analysis: Supporting; Writing – review & editing: Supporting; patient care: Equal). James F. Trotter, MD (Formal analysis: Supporting; Writing – review & editing: Supporting; patient care: Equal). Robert S. Rahimi, MD (Formal analysis: Supporting; Writing – review & editing: Supporting; patient care: Equal). Stephen A. Harrison, MD (Formal analysis: Supporting; Writing – review & editing: Supporting; patient care: Equal). Veeral Ajmera, MD (Formal analysis: Supporting; Writing – review & editing: Supporting; Patient care: Equal). Jeffrey D. Wayne, MD (Formal analysis: Supporting; Writing – review & editing: Supporting; patient care: Equal). Marie O'Farrell, PhD (Data curation: Equal; Formal analysis: Equal; Writing – original draft: Equal; Writing – review & editing: Equal). William McCulloch, MB ChB (Conceptualization: Equal; Formal analysis: Supporting; Methodology: Equal; Writing – original draft: Equal; Writing – review & editing: Equal; patient safety: Lead). Katharine Grimmer, MBA (Conceptualization: Supporting; Data curation: Lead; Formal analysis: Supporting; Methodology: Supporting; Project administration: Equal; Writing – original draft: Equal; Writing – review & editing: Supporting; patient safety: Equal). Mary Rinella, MD (Formal analysis: Supporting; Writing – review & editing: Equal). Vincent Wai-Sun Wong, MD (Formal analysis: Supporting; Writing – review & editing: Supporting). Vlad Ratziu, MD (Formal analysis: Supporting; Writing – review & editing: Equal). Gregory J. Gores, MD (Formal analysis: Supporting; Writing – review & editing: Equal). Brent A. Neuschwander-Tetri, MD (Formal analysis: Supporting; Writing – review & editing: Equal). George Kemble, PhD (Conceptualization: Supporting; Formal analysis: Equal; Funding acquisition: Lead; Project administration: Equal; Supervision: Equal; Writing – original draft: Lead; Writing – review & editing: Equal).

Conflicts of interest
These author disclose the following: Rohit Loomba serves as a consultant or advisory board member for Arrowhead Pharmaceuticals, AstraZeneca, Bird Rock Bio, Boehringer Ingelheim, Bristol-Myer Squibb, Celgene, Cirius, CohBar, Conatus, Eli Lilly, Galmed, Gemphire, Gilead, Glympse bio, GNI, GRI Bio, Intercept, Ionis, Janssen Inc., Merck, Metacrine, Inc, NGM Biopharmaceuticals, Novartis, Novo Nordisk, Pfizer, Prometheus, Sagimet, Sanofi, Siemens, and Viking Therapeutics. In addition, his institution has received grant support from Allergan, Boehringer-Ingelheim, Bristol-Myers Squibb, Cirius, Eli Lilly and Company, Galectin Therapeutics, Galmed Pharmaceuticals, GE, Genfit, Gilead, Intercept, Grail, Janssen, Madrigal Pharmaceuticals, Merck, NGM Biopharmaceuticals, NuSirt, Pfizer, pH Pharma, Prometheus, and Siemens. He is also co-founder of Liponexus, Inc. RL receives funding support from NIDDK P30DK120515. Julio Gutierrez is scientific advisor, consultant or investigator for 3V-Bio, BMS, AbbVie, Access Suport Network, Alexion, Allergan, Dova, Durect, Echosens, Eisai, Genfit, Intercept, MediciNova, Merck, Promethera, Sagimet, Sorrento, and Univision. Stephen A. Harrison: Scientific advisor or consultant for Akero, Alentis, Altimmune, Arrowhead, Axcella, Canfite, Cirius, CiVi Biopharma, Cymabay, Echosens, Fibronostics, Forest Labs, Galectin, Genfit, Gilead, Hepion, HistoIndex, Intercept, Madrigal, Medpace, Metacrine, NGM Bio, Northsea, Novartis, Novo Nordisk, PathAI, Poxel, Liminal, Ridgeline, Sagiment, Terns, Viking, 89 Bio. Stock options: Akero, Cirius, Galectin, Genfit, Hepion, HistoIndex, PathAI, Metacrine, NGM Bio, Northsea. Grant/Research support: Akero, Axcella, BMS, Cirius, CiVi Biopharma, Conatus, Cymabay, Enyo, Galectin, Genentech, Genfit, Gilead, Hepion, Hightide, Intercept, Madrigal, Metacrine, NGM Bio, Novartis, Novo Nordisk, Northsea, Pfizer, Sagimet, Viking. Marie O'Farrell: Employee and shareholder of Sagimet Biosciences. William McCulloch: Employee and shareholder of Sagimet Biosciences. Katharine Grimmer: Compensation and shareholder of Sagimet Biosciences. Mary E. Rinella: Scientific advisor or consultant in the past 36 months to Alnylam, Amgen, Boehringer Ingelheim, Bristol-Myers Squibb, Coherus, Enanta, Fractyl, Genentech, Intercept, Madrigal, Merck, Novo Nordisk, NGM Biopharmaceuticals, Novartis, Pfizer, Prociento, Rivus, Sagimet Bio, Siemens and Terns, Thetis, Viking Therapeutics, 89 Bio. All scientific advising contracts cancelled for 2021. Vincent Wong served as a consultant or advisory board member for 3V-BIO, AbbVie, Allergan, Boehringer Ingelheim, Center for Outcomes Research in Liver Diseases, Echosens, Gilead Sciences, Hanmi Pharmaceutical, Intercept, Inventiva, Merck, Novartis, Novo Nordisk, Perspectum Diagnostics, Pfizer, ProSciento, Sagimet Biosciences, TARGET PharmaSolutions, and Terns; and a speaker for AbbVie, Bristol-Myers Squibb, Echosens, and Gilead Sciences. He has received a grant from Gilead Sciences for fatty liver research. Vlad Ratziu: personal fees from Sagimet, during the conduct of the study; personal fees from Boehringer-Ingelheim, Galmed, Intercept, NGM Bio, Poxel, Novo-Nordisk, and Madrigal; grants from Gilead, outside the submitted work. Gregory Gores: personal fees from Sagimet, outside the submitted work. Brent Neuschwander-Tetri: Advisor or consultant for Alimentiv, Allergan, Alnylam, Amgen, Arrowhead, Axcella, Boehringer Ingelheim, BMS, Coherus, Cymabay, Enanta, Fortress, Genfit, Gilead, High Tide, HistoIndex, Innovo, Intercept, Ionis, LG Chem, Lipocine, Madrigal, Medimmune, Merck, Mirum, NGM, NovoNordisk, Novus Therapeutics, pH-Pharma, Sagimet, Target RWE, 89Bio; Stock options: HepGene; Institutional research grants: Allergan, BMS, Cirius, Enanta, Genfit, Gilead, Intercept, Madrigal, NGM. George Kemble: Employee, board member and shareholder of Sagimet Biosciences. The remaining authors disclose no conflicts.

Funding
This study was funded by Sagimet Biosciences Inc.