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PII: S1542-3565(22)00002-7
DOI: https://doi.org/10.1016/j.cgh.2021.12.044
Reference: YJCGH 58258

To appear in: Clinical Gastroenterology and Hepatology
Accepted Date: 24 December 2021


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Fenofibrate Mitigates Hypertriglyceridemia in Nonalcoholic Steatohepatitis Patients Treated With Cilofexor/Firsocostat

Short Title: Fenofibrate for hypertriglyceridemia in NASH

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Grant Support: Gilead Sciences, Inc.

Abbreviations used in this paper: ACC, acetyl-coenzyme A carboxylase; AE, adverse event; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANGPTL4, angiopoietin-like 4; AST, aspartate aminotransferase; CILO, cilofexor; FABP1, fatty acid binding protein 1; FGF21, fibroblast growth factor 21; FIR, firsocostat; FXR, farnesoid X receptor; IQR, interquartile range; GGT, γ-glutamyl transferase; LDL, low-density lipoprotein; MRE, magnetic resonance elastography; NASH, nonalcoholic steatohepatitis; PPAR, peroxisome proliferator-activated receptor; SREBP-1c, sterol regulatory element-binding transcription factor 1c; TE, transient elastography; Q, quartile; VLDL, very low-density lipoprotein.
Reprint requests

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Conflicts of interest:

RL serves as a consultant to Aardvark Therapeutics, Altimmune, Anylam/Regeneron, Amgen, Arrowhead Pharmaceuticals, AstraZeneca, Bristol-Myers Squibb, CohBar, Eli Lilly, Galmed, Gilead, Glymph bio, Hightide, Inipharma, Intercept, Inventiva, Ionis, Janssen Inc., Madrigal, Metacrine, Inc., NGM Biopharmaceuticals, Novartis, Novo Nordisk, Merck, Pfizer, Sagimet, Theratechnologies, 89 bio, Terns Pharmaceuticals and Viking Therapeutics. In addition his institutions received research grants from Arrowhead Pharmaceuticals, Astrazeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Eli Lilly, Galectin Therapeutics, Galmed Pharmaceuticals, Gilead, Intercept, Hanmi, Intercept, Inventiva, Ionis, Janssen, Madrigal Pharmaceuticals, Merck, NGM Biopharmaceuticals, Novo Nordisk, Merck, Pfizer, Sonic Incytes and Terns Pharmaceuticals. Co-founder of LipoNexus Inc. RL receives funding support from NCATS (5UL1TR001442), NIDDK (U01DK061734, U01DK130190, R01DK106419, R01DK121378, R01DK124318, P30DK120515), NHLBI (P01HL147835), and NIAAA (U01AA029019).


Writing assistance

Writing and editorial assistance was provided by Jonathan Simmons and Geoff Marx of BioScience Communications, New York, NY, funded by Gilead.

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Study design and oversight: Ryan S. Huss, Chuhan Chung, Robert P. Myers.

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Drafting of manuscript: Ryan S. Huss, Chuhan Chung, Robert P. Myers

Critical revision of the manuscript for important intellectual content: All authors.
Abstract

BACKGROUND & AIMS: Patients with advanced fibrosis due to nonalcoholic steatohepatitis (NASH) are at high risk of morbidity and mortality. We previously found that a combination of the farnesoid X receptor agonist cilofexor (CILO) and the acetyl-CoA carboxylase (ACC) inhibitor firsocostat (FIR) improved liver histology and biomarkers in NASH with advanced fibrosis but was associated with hypertriglyceridemia. We evaluated the safety and efficacy of icosapent ethyl (Vascepa®) and fenofibrate to mitigate triglyceride elevations in NASH patients treated with CILO and FIR.

METHODS: NASH patients with elevated triglycerides (≥150 and <500 mg/dL) were randomized to Vascepa 2 g twice daily (n=33) or fenofibrate 145 mg daily (n=33) for 2 weeks, followed by the addition of CILO 30 mg and FIR 20 mg daily for 6 weeks. Safety, lipids, and liver biochemistry were monitored.

RESULTS: All treatments were well tolerated; most treatment-emergent adverse events (AEs) were Grade 1–2 severity and there were no discontinuations due to AEs. At baseline (BL), median (Q1, Q3) triglycerides were similar in the Vascepa and fenofibrate groups (177 [154, 205] vs 190 [144, 258] mg/dL, respectively). Median changes from baseline in triglycerides for Vascepa vs fenofibrate after two weeks of pretreatment were -12 mg/dL (-33, 7; P=0.09) vs -32 mg/dL (-76, 6; P=0.010) and at 6 weeks were +41 mg/dL (16, 103; P<0.001) vs -2 mg/dL (-42, 54; P=0.92). In patients with baseline triglycerides <250 mg/dL, fenofibrate was more effective vs Vascepa in mitigating triglyceride increases after 6 weeks of combination treatment (+6 vs +39 mg/dL); similar trends were observed in patients with baseline triglycerides ≥250 mg/dL (-61 vs +99 mg/dL).
CONCLUSION: In NASH patients with hypertriglyceridemia treated with CILO and FIR, fenofibrate was safe and effectively mitigated increases in triglycerides associated with ACC inhibition. ClinicalTrials.gov NCT02781584.

Keywords: Nonalcoholic Fatty Liver Disease; Peroxisome Proliferator-Activated Receptor-α; Very-Low-Density Lipoprotein
Introduction

Nonalcoholic steatohepatitis (NASH) is a leading cause of end-stage liver disease and liver transplantation in the United States (Chalasani 2018; Goldberg 2017). The pathogenesis of NASH is multifactorial and includes contributions from metabolic and other pro-inflammatory insults that contribute to the development of fibrosis and disease progression (Donnelly 2005; Neuschwander-Tetri 2010). Targeting multiple relevant pathogenic mechanisms through a combination treatment approach may, therefore, provide optimal efficacy in NASH, particularly among patients with advanced fibrosis (Neuschwander-Tetri 2010; Dufour 2020; Esler 2019). In the recent phase 2 ATLAS study, treatment with a combination of the farnesoid X receptor (FXR) agonist cilofexor (CILO) and the acetyl-coenzyme A carboxylase (ACC) inhibitor firsocostat (FIR) for 48 weeks led to improvements in liver histology and multiple clinically relevant biomarkers in patients with bridging fibrosis and compensated cirrhosis due to NASH (Loomba 2021).

Firsocostat is a liver-targeted, small-molecule allosteric inhibitor of ACC (isoforms ACC1 and ACC2). Results from preclinical and clinical studies of FIR support potentially beneficial effects in NASH, including improvements in steatosis, necroinflammatory activity, and fibrosis (Goedeke 2018; Lawitz 2018; Loomba 2018; Loomba 2021). An increase in serum triglycerides has, however, been observed in some patients treated with ACC inhibitors including FIR (Lawitz 2018; Loomba 2018; Kim 2017). Hypertriglyceridemia in the setting of ACC inhibition occurs due to compensatory activation of sterol regulatory element-binding transcription factor 1c (SREBP-1c), the master transcriptional regulator of lipogenesis, as well as reduced clearance of triglyceride-rich, very-low-density lipoprotein (VLDL) particles (Kim
These findings suggest that modalities that suppress SREBP-1c activation and/or enhance VLDL clearance may be a potential therapeutic partner for ACC inhibitors.

Activation of FXR induces a transcriptional program that is best known for regulating bile-acid homeostasis (Chiang 2000; Chiang 2003; Matsubra 2013; Puri 2018). The recognition that bile acids control multiple components of hepatic metabolism led to landmark studies that identified the interactions between bile acids, FXR, and the regulation of hepatocyte lipid metabolism, including FXR-mediated suppression of SREBP-1c (Watanabe 2004).

Thus, one objective of the ATLAS study was to determine whether addition of the FXR agonist cilofexor could mitigate the hypertriglyceridemia associated with ACC inhibition (Loomba 2021). In the 48-week ATLAS trial, 4% (3/78) of patients experienced Grade 3 hypertriglyceridemia in the firsocostat/cilofexor cohort. Considering the wide prevalence of NASH and the associated cardiovascular risk profile in this population (Ballestri 2014; Lazo 2013), additional efforts to mitigate hypertriglyceridemia were undertaken with peroxisome proliferator-activated receptor (PPAR)–α agonists, an established mechanism that suppresses SREBP-1c (Lawitz 2019). Based on these observations, we conducted a randomized trial to evaluate the safety and triglyceride-lowering effects of Vascepa® (icosapent ethyl; Amarin Pharma, Inc., Bridgewater, NJ) or fenofibrate in combination with cilofexor and firsocostat in patients with NASH.

**Materials and Methods**

**Patients and Study Design**

This proof-of-concept, open-label study (ClinicalTrials.gov NCT02781584) was conducted in patients ≥18 years of age with hypertriglyceridemia (serum fasting triglycerides
≥150 and <500 mg/dL), a clinical diagnosis of nonalcoholic fatty liver disease (NAFLD)/NASH, and at least one of the following: ≥2 of 5 criteria for metabolic syndrome (modified from the National Cholesterol Education Program Adult Treatment Panel III guidelines [US Dept of Health and Human Services]); historical liver biopsy consistent with NASH with or without cirrhosis; or liver stiffness by vibration-controlled transient elastography (VCTE) ≥ 9.9 kPa or 2-D magnetic resonance elastography (MRE) ≥ 2.88 kPa, with no documented weight loss >5% between the date of screening and the historical liver biopsy, transient elastography, or MRE. Patients with a history of decompensated liver disease, a Model for End-Stage Liver Disease (MELD) score >12, or a Child-Turcotte-Pugh score >6 were excluded from the study. Full inclusion and exclusion criteria are presented in the Supplementary Materials accompanying this article.

Eligible patients were randomized to receive pretreatment with Vascepa 2 g twice daily (n=33) or fenofibrate 145 mg daily (n=33) for 2 weeks (days -14 thru -1), followed by the addition of CILO 30 mg and FIR 20 mg daily for 6 weeks. All treatments were administered orally (Supplementary Figure 1). Randomization was stratified by serum fasting triglyceride level at screening (≥150 and <250 mg/dL vs ≥250 and <500 mg/dL).

Study Assessments

Safety was assessed by clinical laboratory tests, physical examinations, vital sign measurements, and documentation of adverse events (AEs). Other assessments, including serum lipids, liver biochemistry, and hematology and coagulation parameters, were conducted during the pretreatment phase 2 weeks prior to dosing with Vascepa or fenofibrate, and during the
combination treatment phase at day 1 prior to dosing with CILO and FIR, and at weeks 4 and 6. Posttreatment safety was evaluated via telephone visit at week 8.

**Outcome Measures**

The primary outcome was the safety and tolerability of CILO and FIR in combination, with either Vascepa or fenofibrate, in patients with NASH and hypertriglyceridemia. Exploratory endpoints included changes in fasting serum lipid concentrations, changes from baseline in markers of liver injury and function (alanine aminotransferase [ALT], aspartate aminotransferase [AST], bilirubin, γ-glutamyl transferase [GGT], and alkaline phosphatase [ALP]), biomarkers of PPAR-α agonism (serum angiopoietin-like 4 [ANGPTL4], fatty acid binding protein 1 [FABP1], fibroblast growth factor 21 [FGF21]), and the FGF21-degrading protease fibroblast activation protein (FAP), which were all measured post hoc by enzyme-linked immunosorbent assay.

**Statistical Analyses**

A sample size of 30 patients per cohort was selected to provide 85% power to detect an increase in serum triglycerides from pretreatment baseline to week 6 based on a one-sided t-test at a significance level of 0.05. Differences between pretreatment baseline (day -14) and post-pretreatment values within each treatment group were assessed by Wilcoxon signed-rank test. Between-groups comparisons for relative (percent) change from pretreatment baseline were based on van Elteren test with adjustment for pretreatment baseline serum triglyceride level (< vs ≥250 mg/dL). For demographics and baseline characteristics, differences between groups were analyzed by Fisher’s exact test for categorical variables and Wilcoxon rank-sum test for
continuous variables. All analyses were conducted using SAS® 9.4 (SAS Institute Inc., Cary, NC).

Study Oversight

This study was approved by the institutional review board or independent ethics committees at all participating sites and was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulatory requirements. The study was designed and conducted according to a protocol by the sponsor (Gilead Sciences, Inc., Foster City, CA) in collaboration with the principal investigator (EL). The sponsor collected the data, monitored study conduct, and performed statistical analyses. All authors had access to the data and assume responsibility for the integrity and completeness of the reported data. All authors reviewed and approved the manuscript.

Results

Patient Characteristics and Disposition

Between Nov 19, 2019 and Aug 3, 2020, 123 patients were screened and 66 received treatment at 5 sites in the United States. During the pre-treatment phase, 33 patients received Vascepa and 33 received fenofibrate (Supplementary Figure 2). Demographic and clinical characteristics at pretreatment baseline were similar between groups (Table 1), except for higher median (interquartile range [IQR]) serum ALT and GGT concentrations in patients treated with fenofibrate (ALT: 43 U/L [35, 76]; GGT: 52 U/L [33, 84]) compared with Vascepa (ALT: 29 U/L [22, 44]; GGT: 34 U/L [22, 54]). Of the 66 patients enrolled, 45 qualified based on clinical diagnosis of NASH and metabolic criteria, and 21 were based on historical liver biopsy (n=13;
F0-F1 [n=4], F2-F3 [n=7], F4 [n=2]) or liver stiffness (n=8). Noninvasive tests of fibrosis including FIB-4, APRI, and NAFLD fibrosis score, were similar between groups. At pretreatment baseline, median (IQR) fasting serum triglycerides were 177 mg/dL (154, 205) in the Vascepa group and 190 mg/dL (144, 258) in the fenofibrate group.

In the Vascepa group, 5 of 33 patients (15%) discontinued treatment prematurely: 1 due to an AE of hyperlipidemia during the pretreatment phase (see below) and 4 due to COVID-19-related reasons (2 during the pretreatment phase and 2 during the combination treatment phase). In the fenofibrate group, 1 patient (3%) discontinued treatment due to COVID-19-related issues during the pretreatment phase.

**Safety**

Combination treatments including both CILO and FIR, with either fenofibrate or Vascepa, were well tolerated. Most treatment-emergent AEs were Grade 1–2 in severity and there were no discontinuations due to treatment-emergent AEs (Table 2). Two Grade ≥3 AEs were reported in the fenofibrate group (anemia and appendicitis) and none in the Vascepa group. Two serious AEs were reported, neither attributed to study drug by the investigator, including Grade 1 noncardiac chest pain in a patient in the Vascepa group and Grade 3 appendicitis in a patient in the fenofibrate group, which occurred 9 days after the last dose of study drug.

Prior to initiating CILO and FIR, 1 patient randomized to the Vascepa group with screening and baseline serum triglyceride levels of 277 and 2540 mg/dL, respectively,
discontinued Vascepa after 8 days due to persistent serum triglyceride elevation >500 mg/dL.

During the combination treatment phase, 2 patients in the Vascepa group and 1 in the fenofibrate group experienced Grade 3 elevations in triglycerides (>500–1000 mg/dL). Each of these patients had serum triglycerides ≥250 mg/dL at pretreatment baseline. Grade 2 serum triglyceride elevations (305-478 mg/dL) occurred in 7 patients in the Vascepa group and 4 in the fenofibrate group. All other laboratory abnormalities were Grade ≤2.

**Efficacy**

After 2 weeks of pretreatment with either Vascepa or fenofibrate, median (IQR) changes from baseline in serum triglycerides were -12 mg/dL (-33, 7; P=0.09) and -32 mg/dL (-76, 6; P=0.01), respectively (Figure 1A). During combination treatment with CILO and FIR, median (IQR) changes from baseline in serum triglycerides after 4 and 6 weeks with Vascepa were +28 mg/dL (2, 64; P <0.001) and +41 mg/dL (16, 103; P <0.001), respectively; corresponding figures for fenofibrate were +5 mg/dL (-30, 40; P=0.89) and -2 mg/dL (-42, 54; P=0.92), respectively. In patients with baseline serum triglycerides ≥250 mg/dL, median (IQR) changes from baseline at week 6 for the Vascepa and fenofibrate groups were +99 mg/dL (-29, 185) and -61 mg/dL (-128, -8), respectively (Figure 1B). In patients with baseline serum triglycerides <250 mg/dL, median serum triglycerides increased by 39 mg/dL (IQR 16, 91) among Vascepa-treated patients (P <0.001), but no change was observed among those treated with fenofibrate (+6 mg/dL; IQR -26, 54; P=0.17) (Figure 1C). A significant increase in VLDL from pretreatment baseline at week 6 was observed in the Vascepa group (+8 mg/dL [3, 14]; P <0.001), but not in the fenofibrate group (-1 mg/dL [-7, 11]; p=0.89). In addition, significant decreases in HDL cholesterol from pretreatment baseline at week 6 in the combination treatment phase were observed in both
groups (Vascepa: -7 mg/dL [-13, -3]; \( P < 0.001 \); fenofibrate: -6 mg/dL [-9, 0]; \( P < 0.001 \); Table 3). No significant changes in total or LDL cholesterol were observed.

Improvements in liver biochemistry were observed in both treatment groups; however, significantly greater improvements were observed with fenofibrate versus Vascepa, including for ALT (-37% vs -16%; \( P = 0.002 \)), GGT (-34% vs -13%; \( P = 0.002 \)), and ALP (-14% vs +7%; \( P < 0.001 \); Figure 2). These differences were observed as early as Day 1 prior to dosing with CILO and FIR and continued through weeks 4 and 6 of combination therapy.

Fenofibrate, but not Vascepa, was associated with significant and sustained PPAR-\( \alpha \) engagement as reflected by changes in serum biomarkers directly regulated by PPAR-\( \alpha \), including FGF21, ANGPTL4, and FABP1 (Figure 3). Changes in FAP, a key regulator of FGF21 degradation, were not observed, supporting a PPAR-\( \alpha \)-mediated increase in FGF21 with fenofibrate. Changes in these biomarkers did not differ according to baseline serum triglyceride level (\( \geq 250 \) vs <250 mg/dL) (data not shown).

**Discussion**

In this randomized trial in NASH patients with hypertriglyceridemia (serum triglycerides \( \geq 150 \) and <500 mg/dL), the addition of Vascepa or fenofibrate to a combination of CILO and FIR was safe and well tolerated. Most treatment-emergent AEs were mild to moderate in severity and did not result in premature discontinuation of therapy. Notable is the absence of hepatotoxicity observed in this cohort considering regulatory requirements to include liver disease as a contraindication to fibrate therapy. These results complement a growing body of
literature supporting the safety of fibrates in patients with liver disease, including NASH with advanced (F3–F4) fibrosis (Carrion 2021, Corpechot 2018, Lawitz 2019). Compared with Vascepa, fenofibrate was more effective in mitigating increases in serum triglycerides associated with ACC inhibition. After 6 weeks of combination treatment with CILO and FIR, no change from baseline in serum triglycerides was observed in the fenofibrate group, while an ~22.4% median increase was observed in the Vascepa group. Changes in triglycerides were generally similar in subgroups defined by pretreatment triglyceride level (< or ≥250 mg/dL). Specifically, while an increase in triglycerides was observed in both subgroups among Vascepa-treated patients, those treated with fenofibrate had either no change (with baseline <250 mg/dL) or a nonsignificant decrease (with baseline ≥250 mg/dL) in triglycerides (Figure 1). These are important findings because prior studies have suggested an increased risk of FIR-induced triglyceride elevation in patients with pre-existing hypertriglyceridemia, particularly ≥250 mg/dL (Loomba 2018). Further, the changes in serum triglycerides observed in this study are consistent with prior observations in patients with advanced fibrosis (F3–F4) due to NASH treated with FIR monotherapy (Lawitz 2019). In keeping with prior observations, an increase in VLDL was observed with FIR treatment, but was mitigated with fenofibrate, but not Vascepa, therapy. While small, but statistically significant decreases in HDL were observed in both treatment groups, no significant changes in total or LDL cholesterol occurred after 6 weeks of combination therapy. The absence of significant changes in LDL cholesterol are consistent with prior data for CILO and FIR, both as monotherapy and in combination (Loomba 2018; Loomba 2021; Patel 2020)

Patients with advanced fibrosis due to NASH have an increased risk of both hepatic and cardiovascular complications; hence, consideration of cardiovascular risk factors, including lipid
parameters, is important when evaluating the benefit/risk profile of emerging NASH therapies. Strategies to mitigate potentially adverse lipid changes with novel treatments have been explored, primarily with statins to manage increases in LDL cholesterol (Harrison 2021; Pockros 2019; Rinella 2019). For example, the phase 2 CONTROL study demonstrated the effectiveness of atorvastatin to treat LDL elevations associated with the FXR agonist obeticholic acid (Neuschwander-Tetri 2015; Pockros 2019). Similarly, rosuvastatin has shown promise for the management of increases in LDL that occur with the FGF19 analog aldafermin. (Harrison 2021; Rinella 2019). The rationale behind use of PPAR-α agonist treatment in combination with ACC inhibitor therapy is based on preclinical studies that described the mechanisms that contribute to hypertriglyceridemia in this setting: increased production and secretion of triglyceride-rich VLDL particles from the liver and decreased clearance of triglyceride-rich lipoproteins by lipoprotein lipase (Goedeke 2018; Kim 2017). Triglyceride lowering with fibrates is mediated by PPAR-α-induced stimulation of fatty acid oxidation, increased lipoprotein lipase synthesis, and reduced expression of apolipoprotein C3, which inhibits lipolysis and enhances clearance of VLDL. Use of PPAR-α agonists (eg, fibrates, fish oil) is, therefore, expected to mitigate increases in serum triglycerides resulting from ACC inhibition. Indeed, in preclinical models of NASH and ACC inhibitor therapy and prior clinical studies evaluating FIR (20 mg) as monotherapy in patients with NASH, treatment with PPAR-α agonists in a subset of patients with Grade 3–4 hypertriglyceridemia led to reductions in serum triglycerides toward baseline (Kim 2017; Loomba 2018). In the combination treatment phase of the present study, similar reductions in serum triglycerides toward baseline levels were observed, indicating that fenofibrate treatment may prevent FIR-related triglyceride elevations, both alone and in combination with CILO.
Improvements in liver biochemistry in the present study were similar to those observed with the combination of CILO and FIR in the phase 2 ATLAS study among patients with advanced fibrosis due to NASH (Loomba 2021). Specifically, median relative changes from pretreatment baseline in ALT and AST at week 6 for the fenofibrate combination group were -37.2% and -14.8%, respectively, which are consistent with observations from CILO+FIR–treated patients in ATLAS (Loomba 2021). These results, while over a short treatment duration, suggest that the addition of fenofibrate or Vascepa to CILO+FIR does not mitigate the favorable effects of this combination on liver biochemistry. It should be noted that while greater improvements in liver biochemistry were observed in the fenofibrate vs Vascepa arm at week 6, ALT and GGT were significantly higher in the fenofibrate group at baseline.

The present study was not of sufficient duration to evaluate the impact of fenofibrate or Vascepa on the potential hepatic benefits of CILO+FIR treatment. However, in a separate study in hypertriglyceridemic patients with advanced fibrosis due to NASH randomized to FIR 20 mg in combination with fenofibrate 45 or 145 mg daily for 24 weeks, changes in noninvasive tests of hepatic steatosis and fibrosis were similar to those observed with FIR monotherapy in other studies (Lawitz 2019; Loomba 2018; Loomba 2021). Despite the differing treatment durations between these studies, the combination of FIR and fenofibrate (across both doses) resulted in similar median percent reductions in hepatic steatosis by magnetic resonance imaging–proton density fat fraction at weeks 12 and 24 (-24% and -22%, respectively).

Fenofibrate in combination with CILO and FIR demonstrated significant and sustained PPAR-α engagement as evidenced by increased serum levels of PPAR-α targets, including FGF21, ANGPTL4, and FABP1. Changes in these biomarkers were similar in patients with triglycerides > and <250 mg/dL. PPAR-α plays a central role in fatty acid metabolism; therefore,
successful engagement with its downstream targets highlights the mechanisms by which fenofibrate mitigates ACC-induced elevations in serum triglyceride levels. Other PPAR agonists are being studied as NASH standalone therapy, with mixed findings thus far. In a phase 2 study, PPAR-α/δ dual agonist elafibranor failed to meet its primary efficacy endpoint of NASH resolution (Ratziu 2016). Additionally, the pan-PPAR agonist lanifibranor was evaluated in a phase 2 study and demonstrated efficacy in F2–3 patients on NASH resolution and fibrosis (Francque 2020).

In conclusion, the present study provides both clinical and mechanistic support for the use of fenofibrate in the mitigation of firsocostat-induced serum triglyceride elevations in patients with NASH.

Data Sharing Statement

Gilead shares anonymized Individual Patient Data on request or as required by law and/or regulation with qualified external researchers. Approval of such requests is at Gilead’s discretion and is dependent on the nature of the request, the merit of the research proposed, the availability of the data, and the intended use of the data. Data requests should be sent to datarequest@gilead.com.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of at: xxx.
Acknowledgments

This study was supported by Gilead Sciences. Editorial support was provided by Jonathan Simmons and Geoff Marx of BioScience Communications, New York, NY, and funded by Gilead. We thank the patients and their families, as well as the investigators and site personnel who participated in this study.
References


Table 1. Demographics and Baseline Disease Characteristics

<table>
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<th>Fenofibrate + cilofexor + firsoxostat (n = 32)</th>
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<tr>
<td><strong>ALP, U/L</strong></td>
<td>92 (69–120)</td>
<td>84 (71–101)</td>
</tr>
<tr>
<td><strong>Total bilirubin, mg/dL</strong></td>
<td>0.4 (0.3–0.7)</td>
<td>0.3 (0.2–0.5)</td>
</tr>
<tr>
<td><strong>MELD score</strong></td>
<td>6 (6–7)</td>
<td>6 (6–6)</td>
</tr>
<tr>
<td><strong>APRI</strong></td>
<td>0.3 (0.2–0.6)</td>
<td>0.4 (0.2–0.7)</td>
</tr>
<tr>
<td><strong>FIB-4</strong></td>
<td>1.13 (0.82–1.79)</td>
<td>1.08 (0.74–1.40)</td>
</tr>
<tr>
<td><strong>NFS</strong></td>
<td>-0.611 (-0.767–0.673)</td>
<td>-0.593 (-2.203–0.256)</td>
</tr>
</tbody>
</table>

**NOTE:** All data are n (%) or median (IQR); data represent pretreatment baseline values defined as last available value collected on or prior to first dosing date of Vascepa or fenofibrate.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; APRI, aspartate aminotransferase (AST):platelet ratio index; BMI, body mass index; FIB-4, Fibrosis 4; GGT, γ-glutamyl transferase; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MELD, Model for End-Stage Liver Disease; NFS, nonalcoholic fatty liver disease fibrosis score; TGs, triglycerides; VLDL, very low-density lipoprotein.
<table>
<thead>
<tr>
<th>Overall treatment-emergent AEs&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Vascepa + cilofexor + firsocostat (n = 30)</th>
<th>Fenofibrate + cilofexor + firsocostat (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any AE</td>
<td>12 (40)</td>
<td>12 (38)</td>
</tr>
<tr>
<td>AEs in &gt;5% of patients in either group (preferred term)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthralgia</td>
<td>0</td>
<td>3 (9)</td>
</tr>
<tr>
<td>GERD</td>
<td>0</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Back pain</td>
<td>0</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Fall</td>
<td>0</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Grade ≥3 AE</td>
<td>0</td>
<td>2 (6)</td>
</tr>
<tr>
<td>AE related to study drug</td>
<td>4 (13)</td>
<td>0</td>
</tr>
<tr>
<td>Any serious AE</td>
<td>1 (3)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Serious AE related to study drug</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AE leading to discontinuation of study drug</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AE leading to modification/interruption of study drug</td>
<td>2 (7)&lt;sup&gt;b&lt;/sup&gt; *</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Study 1</td>
<td>Study 2</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Grade 3/4 laboratory abnormalities</td>
<td>2 (7)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Hypertriglyceridemia (Grade 3)</td>
<td>2 (7)</td>
<td>2 (6)</td>
</tr>
</tbody>
</table>

NOTE: All data are n (%).

AE, adverse event; GERD, gastroesophageal reflux disease.

*Treatment-emergent AEs were observed on or after the Vascepa or fenofibrate start date through 30 days after permanent discontinuation of any study drug.

†Noncardiac chest pain and hemarthrosis.

‡All episodes occurred during combination treatment phase, except for 1 patient in the fenofibrate group who experienced Grade 3 hypertriglyceridemia during pretreatment phase.
Table 3. Median Changes in Lipid Parameters From Pretreatment Baseline at Week 6

<table>
<thead>
<tr>
<th></th>
<th>Vascepa + cilofexor + firsocostat (n = 30)</th>
<th>Fenofibrate + cilofexor + firsocostat (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/dL (IQR)</td>
<td>mg/dL (IQR)</td>
</tr>
<tr>
<td>TGs</td>
<td>41 (16, 103)</td>
<td>-2 (-42, 54)</td>
</tr>
<tr>
<td>P value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>0.92</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>8 (-12, 22)</td>
<td>8 (-19, 21)</td>
</tr>
<tr>
<td>P value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19</td>
<td>0.57</td>
</tr>
<tr>
<td>HDL</td>
<td>-7 (-13, -3)</td>
<td>-6 (-9, 0)</td>
</tr>
<tr>
<td>P value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL</td>
<td>-1 (-12, 14)</td>
<td>8 (-14, 15)</td>
</tr>
<tr>
<td>P value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.75</td>
<td>0.39</td>
</tr>
<tr>
<td>VLDL</td>
<td>8 (3, 14)</td>
<td>-1 (-7, 11)</td>
</tr>
<tr>
<td>P value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>0.89</td>
</tr>
</tbody>
</table>

NOTE: All data are median (interquartile range).

HDL, high-density lipoprotein; LDL, low-density lipoprotein; NS, not significant; TGs, triglycerides; VLDL, very low-density lipoprotein.

<sup>a</sup>Wilcoxon signed-rank test comparing pretreatment baseline (week -2) with week-6 value.
Figure Legend

**Figure 1.** Median (interquartile range [IQR]) changes from pretreatment baseline in fasting serum triglycerides in patients treated with Vascepa (VAS) or fenofibrate (FENO) alone and in combination with cilofexor (CILO) and firsocostat (FIR) in (A) all patients, (B) patients with pretreatment serum triglycerides <250 mg/dL, and (C) patients with pretreatment serum triglycerides ≥250 mg/dL. Shaded areas indicate 2-week pretreatment phase with VAS or FENO monotherapy. Wilcoxon signed-rank test used to compare pretreatment baseline (day -14) with post-pretreatment baseline values. *P = .01; †P < .001.

**Figure 2.** Median (interquartile range [IQR]) percent changes from pretreatment baseline in liver biochemistry in patients treated with Vascepa (VAS) or fenofibrate (FENO) in combination with cilofexor (CILO) and firsocostat (FIR) through week 6. Shaded areas indicate 2-week pretreatment phase with VAS or FENO monotherapy. P values comparing treatment groups are based on van Elteren test by adjusting pretreatment baseline serum triglyceride level (< vs ≥250 mg/dL). ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase. *P < .05; †P < .001.

**Figure 3.** Median (interquartile range [IQR]) relative (percent) changes from pretreatment baseline in biomarkers of peroxisome proliferator-activated receptor-α (PPAR-α) engagement in patients treated with Vascepa (VAS) or fenofibrate (FENO) in combination with cilofexor (CILO) and firsocostat (FIR) through week 6, including PPAR-α targets fibroblast growth factor 21 (FGF21), angiopoietin-like 4 (ANGPTL4), and fatty-acid binding protein 1 (FABP1), and the FGF21-degrading protease fibroblast activation protein (FAP). Shaded areas indicate the 2-week
pretreatment phase with VAS or FENO monotherapy. *$P < .05$, Wilcoxon signed-rank test used to compare posttreatment with baseline values. #$P < .05$, Wilcoxon rank-sum test used to compare VAS and FENO treatment groups.
B  

Baseline triglycerides <250 mg/dL

- VAS + CILO + FIR (n = 26)
- FENO + CILO + FIR (n = 22)

Pretreatment phase

CILO + FIR Combination phase

Median change in serum triglycerides from pretreatment baseline, mg/dL (IQR)

Week

-2 0 2 4 6

-150 -100 -50 0 50 100 150 200

-11 -29 28 39

†
Baseline triglycerides ≥250 mg/dL

- Pretreatment phase
  - VAS + CILO + FIR (n = 4)
  - FENO + CILO + FIR (n = 10)

- CILO + FIR Combination phase
  - VAS + CILO + FIR (n = 4)
  - FENO + CILO + FIR (n = 10)

Week
-2 0 2 4 6

Changes in triglyceride levels over time for different treatment groups.
4. SUBJECT POPULATION

4.2. Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for participation in this study.

1. Males and females ≥ 18 years of age; inclusive based on the date of the Screening Visit;
2. Willing and able to provide informed consent prior to any study specific procedures being performed

6. Subjects must have a clinical diagnosis of NAFLD/NASH and the following criteria:

   a) At least two criteria for metabolic syndrome modified from the NCEP ATP III Guidelines, at Screening:
      i. Fasting glucose ≥ 100 mg/dL or receiving drug treatment for elevated glucose,
      ii. Fasting HDL cholesterol < 40 mg/dL in men and < 50 mg/dL in women or receiving drug treatment for low HDL cholesterol,
      iii. Fasting triglycerides ≥ 150 mg/dL,
      iv. Waist circumference ≥ 102 cm for men or ≥ 88 cm for women or BMI ≥ 30 kg/m2,
      v. Systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or receiving drug treatment for hypertension,

 OR one of the following criteria:

   b) A historical liver biopsy within 6 months of Screening consistent with NASH for subjects without compensated cirrhosis (F4); or within 12 months of Screening consistent with NASH for subjects with compensated cirrhosis (F4) in the opinion of the investigator,
   c) A historical MRE with liver stiffness ≥ 2.88 kPa within 6 months of Screening,
   d) A historical FibroScan® with liver stiffness ≥ 9.9 kPa within 6 months of Screening,
   AND
   e) No documented weight loss > 5% between the date of the historical liver biopsy,
7. A Platelet count ≥ 100,000/μL;
8. [applicable only to COHORTS 1-9]
9. Estimated glomerular filtration rate (eGFR) ≥ 60 mL/min as calculated by the Cockcroft-Gault equation at Screening;
10. Serum triglyceride level ≥ 150 mg/dL at Screening;
11. Female subjects of childbearing potential (see definition in Appendix 3) must have a negative serum pregnancy test prior to starting study treatment;
12. All female subjects of childbearing potential who engage in heterosexual intercourse must agree to use a highly effective method of contraception during intercourse from the screening visit throughout the study period and for 90 days following the last dose of study drug as described in Appendix 3;
13. Male subjects must agree to use condoms during intercourse from screening through the study completion and for 90 days following the last dose of study drug;
14. Male subjects must refrain from sperm donation from screening through at least 90 days following the last dose of study drug;
15. Female subjects must refrain from egg donation or harvest for 90 days after last dose of study drug;
16. [applicable only to COHORTS 1-11]
17. Willing and able to comply with scheduled visits, drug administration plan, laboratory tests, and other study procedures and study restrictions.

4.3. Exclusion Criteria
Subjects who meet any of the following exclusion criteria are not to be enrolled in this study.
1. Pregnant or lactating females;
2. Other causes of liver disease including autoimmune, viral, and alcoholic liver disease;
3. Any history of decompensated liver disease, including ascites, hepatic encephalopathy, or variceal bleeding;
4. Child-Pugh-Turcotte (CPT) score > 6 (Appendix 4) at Screening, unless due to an alternative etiology such as Gilbert’s syndrome or therapeutic anticoagulation;
5. History of liver transplantation;
6. History of hepatocellular carcinoma;
7. Weight reduction surgery in the past 2 years or planned during the study;
8. Documented weight loss > 5% between the date of the historical liver biopsy and Screening, if applicable;
9. BMI < 18 kg/m²;
10. ALT > 5 x ULN at Screening;
11. HbA1c ≥ 9.5% (or serum fructosamine ≥ 381 μmol if HbA1c is unable to be resulted) at Screening;
12. Hemoglobin ≤ 10.6 g/dL at Screening;
13. INR > 1.4 at Screening, unless on anticoagulation therapy;
14. Total bilirubin > 1.3 x ULN except in confirmed cases of Gilbert’s syndrome;
15. Triglycerides ≥ 500 mg/dL at Screening;
16. Model for End-Stage Liver Disease (MELD) score > 12 at Screening, unless due to an alternate etiology such as therapeutic anticoagulation;
17. Chronic hepatitis B (HBsAg positive);
18. Chronic hepatitis C (HCV RNA positive); Subjects cured of HCV infection less than 2 years prior to the Screening visit are not eligible;
19. HIV Ab positive;
20. Presence of gallstones within 6 months of Screening;
21. Alcohol consumption greater than 21 oz/week for males or 14 oz/week for females (1oz/30mL of alcohol is present in 1 12oz/360mL beer, 1 4oz/120mL glass of wine, and a 1 oz/30mL measure of 40% proof alcohol);
22. Positive urine screen for amphetamines, cocaine or opiates (i.e., heroin, morphine) at Screening. Subjects on stable methadone or buprenorphine maintenance treatment for at least 6 months prior to screening may be included in the study. Subjects with a positive urine drug screen due to prescription opioid-based medication are eligible if the prescription and diagnosis are reviewed and approved by the investigator;
23. Unstable cardiovascular disease as defined by any of the following:
   a) Unstable angina within 6 months prior to screening,
   b) Myocardial infarction, coronary artery bypass graft surgery or coronary angioplasty within 6 months prior to screening,
c) Transient ischemic attack or cerebrovascular accident within 6 months prior to screening,
d) Obstructive valvular heart disease or hypertrophic cardiomyopathy,
e) Congestive heart failure;

24. History of intestinal resection of the extent that would result in malabsorption;
25. Use of any prohibited concomitant medications as described in Section 5.8;
26. History of a malignancy within 5 years of screening with the following exceptions:
   a) Adequately treated carcinoma in situ of the cervix,
   b) Adequately treated basal or squamous cell cancer or other localized non-melanoma skin cancer;
27. Any laboratory abnormality or condition that, in the investigator’s opinion, could adversely affect the safety of the subject or impair the assessment of study results;
28. Participation in another investigational study of a drug or device within 1 month prior or within 5 half-lives of the prior investigational agent (whichever is longer) prior to Screening;
29. Concurrent participation in another therapeutic clinical study;
30. Known hypersensitivity to study drugs, the metabolites, or formulation excipients;
31. Presence of any condition that could, in the opinion of the investigator, compromise the subject’s ability to participate in the study, such as history of substance abuse or a psychiatric or medical condition;
32. Unavailable for follow-up assessment or concern for subject’s compliance with the protocol procedures;
33. [applicable only to COHORTS 1-11]
34. Any contraindication to fenofibrate, per the approved package insert, with the exception of advanced liver fibrosis;
35. Any contraindication to Vascepa®, per the approved package insert;
36. History of acute or chronic pancreatitis;
37. Known hypersensitivity to fish and/or shellfish;
38. Poorly controlled hypertension despite anti-hypertensive therapy.
**Supplementary Figure 1.** Study design. CILO, cilofexor; FENO, fenofibrate; FIR, firsocostat; VAS, Vascepa.
**Supplementary Figure 2.** Patient flow diagram. CILO, cilofexor; FENO, fenofibrate; FIR, firsocostat; VAS, Vascepa.
Fenofibrate Mitigates Hypertriglyceridemia in Nonalcoholic Steatohepatitis Patients Treated With Cilofexor/Firsocostat

What You Need to Know

BACKGROUND: Treatment of NASH with advanced fibrosis using cilofexor and firsocostat has demonstrated improvements in liver histology and noninvasive markers. Firsocostat, an inhibitor of acetyl-CoA carboxylase, leads to elevated serum triglycerides in some patients. We tested whether the addition of fenofibrate or icosapent ethyl (Vascepa) to cilofexor and firsocostat would be effective in mitigating hypertriglyceridemia.

FINDINGS: Treatments were well tolerated. Fenofibrate, but not icosapent ethyl, initiated prior to cilofexor and firsocostat effectively mitigated any increase in serum triglycerides. PPAR-α target engagement was more pronounced with fenofibrate than icosapent ethyl.

IMPLICATIONS FOR PATIENT CARE: Fenofibrate can mitigate hypertriglyceridemia in NASH patients treated with cilofexor and firsocostat.