Research Article
NAFLD and Alcohol-Related Liver Diseases

JOURNAL OF HEPATOLOGY

Hepatic and renal improvements with FXR agonist vonafexor in individuals with suspected fibrotic NASH

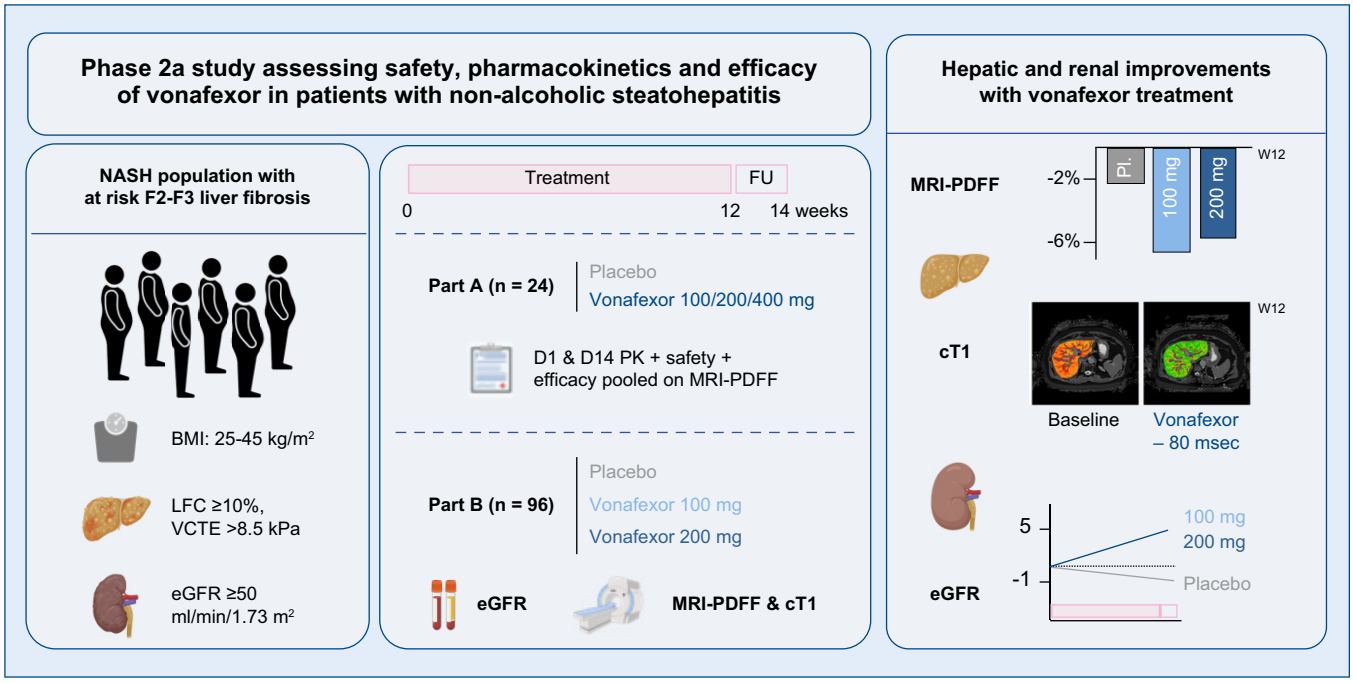
Authors

Vlad Ratziu, Stephen A. Harrison, Véronique Loustaud-Ratti, …, Arun Sanyal, Jacky Vonderscher, Pietro Scalfaro

Correspondence

vlad.ratziu@inserm.fr (V. Ratziu), ps@enyopharma.com (P. Scalfaro).

Graphical abstract



Highlights

- Vonafexor is an FXR agonist in development for patients with NASH and at-risk liver fibrosis.
- In this randomized trial, vonafexor was safe, induced liver fat reduction and weight loss, and improved liver enzymes and renal function.
- As NASH increases the risk of kidney problems, these results support development of vonafexor for patients with liver and kidney disease.

Impact and implications

Non-alcoholic steatohepatitis (NASH) has become a leading cause of chronic liver disease worldwide. Affected patients are also at higher risk of developing chronic kidney disease. There are no approved therapies and only few options to treat this population. The phase IIa LIVIFY trial results show that single daily administration of oral vonafexor, an FXR agonist, leads in the short term to a reduction in liver fat, liver enzymes, fibrosis biomarkers, body weight and abdominal circumference, and a possible improvement in kidney function, while possible mild moderate pruritus (a peripheral FXR class effect) and an LDL-cholesterol increase are manageable with lower doses and statins. These results support exploration in longer and larger trials, with the aim of addressing the unmet medical need in NASH.

https://doi.org/10.1016/j.jhep.2022.10.023
 J. Hepatol. 2023, 78, 479–492

Research Article
NAFLD and Alcohol-Related Liver Diseases

JOURNAL
OF HEPATOLOGY

Hepatic and renal improvements with FXR agonist vonafexor in individuals with suspected fibrotic NASH

Vlad Ratziu[1,*], Stephen A. Harrison[2], Véronique Loustaud-Ratti[3], Christophe Bureau[4,5], Eric Lawitz[6,7], Manal Abdelmalek[8], Naim Alkhouri[9], Sven Francque[10], Hugo Girma[11], Raphaël Darteil[11], Harold Couchoux[11], Myles Wolf[12], Arun Sanyal[13], Jacky Vonderscher[11], Pietro Scalfaro[11,*]

Journal of Hepatology **2023**. vol. 78 | 479–492

Background & Aims: The LIVIFY trial investigated the safety, tolerability, and efficacy of vonafexor, a second-generation, non-bile acid farnesoid X receptor agonist in patients with suspected fibrotic non-alcoholic steatohepatitis (NASH).
Methods: This double-blind phase IIa study was conducted in two parts. Patients were randomised (1:1:1:1) to receive placebo, vonafexor 100 mg twice daily (VONA-100BID), vonafexor 200 mg once daily (VONA-200QD), or 400 mg vonafexor QD (VONA-400QD) in Part A (safety run-in, pharmacokinetics/pharmacodynamics) or placebo, vonafexor 100 mg QD (VONA-100QD), or VONA-200QD (1:1:1) in Part B. The primary efficacy endpoint was a reduction in liver fat content (LFC) by MRI-proton density fat fraction, while secondary endpoints included reduced corrected T1 values and liver enzymes, from baseline to Week 12.
Results: One hundred and twenty patients were randomised (Part A, n = 24; Part B, n = 96). In Part B, there was a significant reduction in least-square mean (SE) absolute change in LFC from baseline to Week 12 for VONA-100QD (-6.3% [0.9]) and VONA-200QD (-5.4% [0.9]), *vs*. placebo (-2.3% [0.9], $p = 0.002$ and 0.012, respectively). A >30% relative LFC reduction was achieved by 50.0% and 39.3% of patients in the VONA-100QD and VONA-200QD arms, respectively, but only in 12.5% in the placebo arm. Reductions in body weight, liver enzymes, and corrected T1 were also observed with vonafexor. Creatinine-based glomerular filtration rate improved in the active arms but not the placebo arm. Mild to moderate generalised pruritus was reported in 6.3%, 9.7%, and 18.2% of participants in the placebo, VONA-100QD, and VONA-200QD arms, respectively.
Conclusions: In patients with suspected fibrotic NASH, vonafexor was safe and induced potent liver fat reduction, improvement in liver enzymes, weight loss, and a possible renal benefit.
Clinical trial number (EudraCT): 2018-003119-22.
ClinicalTrials.gov Identifier: NCT03812029.

Introduction

Non-alcoholic steatohepatitis (NASH) is a progressive form of non-alcoholic fatty liver disease (NAFLD) marked by varying levels of steatosis, hepatocellular damage, inflammation, and fibrosis that can progress to cirrhosis, liver failure, and hepatocellular carcinoma if uncontrolled.[1] The prevalence of NASH has increased over the past decade making it one of the most common causes of liver transplantation in the USA.[2–4] Common comorbidities include obesity, type 2 diabetes mellitus (T2DM), hypertension, and cardiac diseases. NAFLD and NASH are also associated with an increased risk of chronic kidney disease (CKD).[5,6] The presence of NAFLD is associated with higher rates of CKD and increased fibrosis on kidney biopsy, even after controlling for common risk factors such as T2DM, hypertension, and obesity.[5,7–9] There are currently no drugs approved for the treatment of NASH.[10]

The farnesoid X receptor (FXR) is a nuclear hormone receptor that is highly expressed in the liver, intestine, kidney, and to a lesser extent in adrenal glands, and cardiovascular tissue.[11,12] Primary functions of FXR include bile acid homeostasis and regulation of bile acid biosynthesis from cholesterol. However, FXR also affects glucose and lipid metabolism, oxidative stress, inflammation, and the microbiome. Treatment with FXR agonists can induce histological improvement of hepatic fibrosis and other NASH-related histological lesions.[13,14] However, pruritus,[13] increases in LDL-cholesterol,[15] and increased bile lithogenicity[16] limit clinical application of existing FXR agonists. There remains an unmet need for FXR agonists that maintain the histological benefits while minimizing side effects.[10]

Keywords: NASH; farnesoid X receptor; liver fat reduction; MRI-PDFF; eGFR; steatosis; randomised clinical trial; ALT; fibrosis.
Received 22 August 2022; received in revised form 4 October 2022; accepted 21 October 2022; available online 9 November 2022
* Corresponding authors. Addresses: Hôpital de la Pitié-Salpêtrière, 47-83 Boulevard de l'Hôpital, Paris, 75013; France. (V. Ratziu), or ENYO Pharma SA, 60 Avenue Rockefeller, F-69008 Lyon, France. (P. Scalfaro).
E-mail addresses: vlad.ratziu@inserm.fr (V. Ratziu), ps@enyopharma.com (P. Scalfaro).
https://doi.org/10.1016/j.jhep.2022.10.023

Vonafexor (EYP001a) is a second-generation, synthetic, non-steroidal, non-bile salt, orally active carboxylic acid FXR agonist that is currently under development for the treatment of chronic liver diseases. *In vitro*, vonafexor is a potent and highly selective FXR agonist, and early clinical data have shown good efficacy and safety at oral doses of up to 500 mg QD. The LIVIFY study presented herein investigated safety, tolerability and efficacy of vonafexor compared with placebo in patients with either biopsy-confirmed NASH fibrosis or suspected NASH with fibrosis as determined by non-invasive testing.

Patients and methods

Study design and participants

This phase IIa, randomised, double-blind, multicentre, placebo-controlled study was conducted in two parts (Part A and Part B) at 40 sites across the USA and Europe to evaluate the safety and efficacy of vonafexor in men and women aged 18 years or older with fibrotic NASH, diagnosed histologically or clinically. Histological diagnosis of fibrotic NASH, when available from history (within 12 months of screening), was based on a liver biopsy documenting steatohepatitis and fibrosis stage 2 or 3 according to the NASH CRN classification.[17] In the absence of a liver biopsy, a clinical diagnosis of fibrotic NASH was defined as liver stiffness ≥8.5 kPa (FibroScan® vibration-controlled transient elastography [VCTE], compatible with liver fibrosis stage 2 or 3), FibroScan controlled attenuation parameter compatible with the presence of steatosis (*i.e.* >300 dB/m), LFC of ≥10% (measured by MRI-PDFF), and exclusion of non-NASH liver disease. Patients were excluded from the study if they had a BMI >45 kg/m^2, type 1 diabetes, a history of clinically significant cardiovascular or cerebrovascular disease (within 90 days of first drug administration), were immunocompromised, or had a history of cirrhosis or liver decompensation, alanine aminotransferase (ALT) >5x the upper limit of normal (ULN), or aspartate aminotransferase (AST) >5x ULN. Full inclusion and exclusion criteria are provided in the study protocol.

Part A was comprised of a 12-week treatment period, which included a 28-day safety run-in period with intense pharmacokinetic/pharmacodynamic (PK/PD) monitoring on Days 1 and 14 and a 2-week safety follow-up period (Fig. 1). Part B was initiated following completion of an unblinded review of Part A by an external, independent Data Safety Monitoring Committee. Part B comprised a 12-week treatment period and a 2-week safety follow-up (Fig. 1). The primary endpoint was assessed separately at Week 12 for Part A and Part B.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, the International Council for Harmonisation Guidelines for Good Clinical Practice, and with local regulatory requirements. All patients included in the study provided written informed consent.

Sample size

Sample size for Part A was determined using an empirical PK/PD sample size estimate of n = 6 per arm. The sample size of Part B was determined based on the primary efficacy endpoint of absolute change in LFC from baseline to Week 12. It was assumed that the treatment difference between each active treatment arm and placebo was at least 5.1% with a common SD of 5.8%. An estimated sample size of 30 patients per treatment arm was considered sufficient based on a 2-sample Z-test with the power of 0.8, and overall family-wise 1-sided alpha of 0.025 (or each active *vs.* placebo alpha = 0.0125 after Bonferroni adjustment). Calculations also accounted for an approximate dropout rate of 13%.

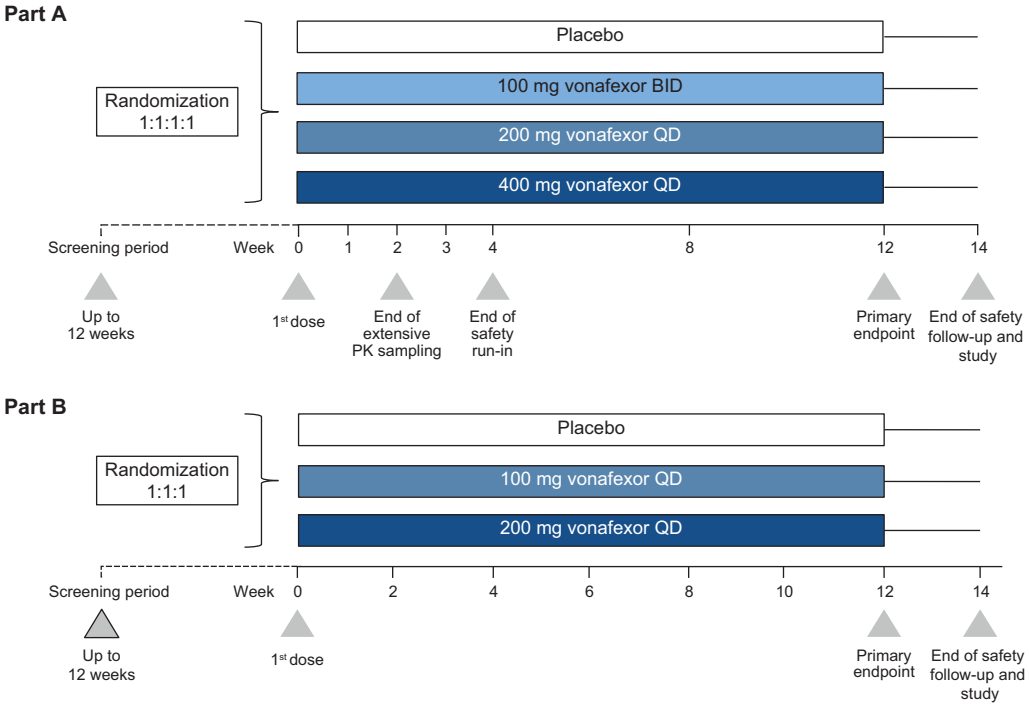


Fig. 1. Design schematic for Part A and Part B. BID, twice daily; PK, pharmacokinetic; QD, once daily.

Randomisation and procedures

In Part A, patients were randomised centrally (1:1:1:1) by interactive response technology to receive placebo, vonafexor 100 mg twice daily (VONA 100BID), vonafexor 200 mg once daily (VONA 200QD), or 400 mg vonafexor QD (VONA 400QD), stratified by statin use and T2DM status.

In Part B, patients were randomised centrally (1:1:1) by interactive response technology to receive placebo QD, VONA-100QD, or VONA-200QD (100 mg of vonafexor for the first 2 weeks, and 200 mg thereafter), stratified by statin use and LFC (LFC <16%, 16%≤ LFC <22%, and 22%≤ LFC).

The study team, patients, investigators, and all clinical site personnel were blinded for the duration of the trial. Two planned Data Safety Monitoring Committee interim analyses were conducted by an independent unblinded statistician to maintain the study blind; the first (inclusive of all available safety and PK/PD data from the Part A safety run-in cohort) was conducted before treatment was initiated in Part B, the second was performed when 50% of patients had completed Week 8 of Part B.

LFC and iron-corrected T1 (cT1) MRI imaging were measured at screening and at Week 12 or end of treatment in Part A and Part B, as part of the primary and secondary efficacy assessments using standardised imaging protocols.[18–20] Body measurements and blood samples for lipid and metabolic profiling were obtained at scheduled visits in Part A and Part B. Biochemical markers of liver fibrosis and inflammation included ALT, AST, AST/ALT ratio, gamma-glutamyl transferase (GGT), adiponectin, high-sensitivity C-reactive protein, interleukin 6, tumour necrosis factor-alpha, cytokeratin-18, fibronectin, hyaluronic acid, procollagen type III N-terminal peptide, tissue inhibitor of metalloproteinases-1 (and derived enhanced liver fibrosis score), Pro-C3, chitinase-3-like protein 1 (also known as YKL-40) and derived Fibrotest and Fibrometer scores (both *post hoc*).

Clinical safety laboratory parameters (chemistry, haematology, and coagulation) and other safety parameters were assessed at all scheduled visits. Kidney parameters included estimated glomerular filtration rate (eGFR [ml/min/1.73m^2] assessed by the Modification of Diet in Renal Disease formula), blood urea nitrogen (mg/dl) and uric acid (mmol/L). Adverse events of special interest (AESIs) included muscle-related adverse events (AEs), drug-induced liver injury (DILI) and pruritus (assessed using a visual analogue scale and the 5-D [degree, duration, direction, disability, and distribution] itch scale).[21,22]

PK/PD profiles were assessed during the Part A safety run-in. PK sampling took place on Day 1 and Day 14. PD markers were 7-α-hydroxy-4-cholesten-3-one (C4) and fibroblast growth factor 19 (FGF19). Sampling was conducted in a fasted state from 0 h (pre-dose) to 4 h post-dose, inclusive. Vonafexor, FGF19 and C4 concentrations were determined using validated bioanalytical methods in accordance with good laboratory practices.

Outcomes

All endpoints were assessed separately for Part A and Part B. The primary efficacy endpoint in Part A and Part B was the absolute change in LFC from baseline to Week 12. Key secondary efficacy endpoints included: absolute and relative reduction from baseline in LFC at Week 12; change in MRI-derived cT1; changes in liver elastography (by FibroScan VCTE), BMI and other body parameters from baseline to Week 12.

Safety and tolerability assessments included monitoring of AEs, findings from physical examinations, vital signs, 12-lead electrocardiogram, and clinical safety laboratory parameters. Coadministration of vonafexor with statins, the impact of treatment on lipid and metabolic profiles, biomarkers of liver fibrosis, inflammation and kidney parameters were also assessed.

Statistical analysis

In Part A, analysis of LFC and cT1 was performed using the modified intent-to-treat (mITT) population (patients who had valid baseline and Week 12 or early termination measurements of LFC), all other analyses were performed using the ITT or safety populations, as applicable. An analysis of covariance (ANCOVA) model was used with baseline fat fraction as a covariate, and treatment, statin use, and T2DM status as factors. Due to the small sample size all study efficacy endpoints were assessed for Vonafexor pooled (n = 17) *vs.* placebo (n = 7).

In Part B, primary and secondary efficacy analyses are presented by treatment arm. Analysis on LFC and cT1 were performed using the mITT population. Statin use and LFC at screening were included as factors in the ANCOVA model with pairwise treatment comparisons least-square (LS) means, SE, 95% CI, and *p* values presented. The Bonferroni method was used to adjust the three tests for multiplicity and to control the 0.05 and two-sided family-wise type I error. All analyses were also performed on the ITT population showing similar results but are not presented here.

All secondary efficacy variables were summarised using descriptive statistics and were performed on the ITT population. Statistical analysis based on change from baseline to Week 12 or Week 14, was conducted using an ANCOVA model. Variables measured at multiple timepoints (eGFR, apolipoprotein B, serum cholesterol, HDL-cholesterol, LDL-cholesterol, lipoprotein-a, and haemoglobin A1c; Wilcoxon Rank-Sums analysis was performed on triglycerides) were analysed using a mixed model for repeated measures. Responder (categorical) variables were analysed using a Cochran-Mantel-Haenszel test.

Analysis of responder variables was repeated using logistic regression models, adjusting for treatment, statin use, and T2DM status at screening in Part A, and by treatment and statin use in Part B.

Safety analysis was conducted on the safety population (patients administered at least one dose of study drug).

Results

Study participants were enrolled between 30 January 2019 and 24 March 2021.

Part A – safety run-in, PK and efficacy of vonafexor in patients with NASH

In Part A, 24 of the 153 patients screened were randomised to one of the four parallel treatment arms forming the safety run-in cohort (vonafexor pooled: n = 17, placebo: n = 7) (Fig. S3). Seven of 17 (41.2%) patients in the vonafexor arms, and 5/7 (71.4%) patients in the placebo arm completed Part A. Nine patients treated with vonafexor discontinued due to an AE.

Table 1. Demographic and baseline characteristics (Part A and Part B).

	Part A		Part B		
	Placebo (n = 7)	Vonafexor pooled (n = 17)	Placebo QD (n = 32)	Vonafexor 100 mg QD (n = 31)	Vonafexor 200 mg QD (n = 33)
Age (years), mean (SD)	48.7 (18.4)	56.1 (8.9)	57.3 (10.3)	58.1 (13.7)	54.0 (11.9)
Sex, n (%)					
Male	5 (71.4)	3 (17.6)	14 (43.8)	17 (54.8)	12 (36.4)
Female	2 (28.6)	14 (82.4)	18 (56.3)	14 (45.2)	21 (63.6)
Ethnicity, n (%)					
Hispanic or Latino	2 (28.6)	5 (29.4)	6 (18.8)	8 (25.8)	10 (30.3)
Not Hispanic or Latino	5 (71.4)	12 (70.6)	26 (81.3)	23 (74.2)	23 (69.7)
Race, n (%)					
American Indian or Alaska Native	0	0	0	0	1 (3.0)
Black or African American	0	1 (5.9)	3 (9.4)	2 (6.5)	1 (3.0)
White	7 (100.0)	16 (94.1)	29 (90.6)	26 (83.9)	31 (93.9)
Other	0	0	0	3 (9.7)	0
BMI (kg/m^2), mean (SD)	39.30 (10.2)	38.78 (9.0)	34.31 (4.3)	34.26 (4.1)	35.40 (5.1)
Waist circumference (cm), mean (SD)	128.9 (31.5)	116.1 (16.0)	112.6 (13.0)	111.4 (8.6)	114.1 (11.8)
NASH comorbidities, n (%)					
Type 2 diabetes	4 (57.1)	10 (58.8)	19 (59.4)	15 (48.4)	13 (39.4)
Hypertension	3 (42.9)	15 (88.2)	19 (59.4)	22 (71.0)	18 (54.5)
Statin use	4 (57.1)	9 (52.9)	11 (34.4)	11 (35.5)	12 (36.4)
Liver histology fibrosis stage#					
1	0	0	2 (6.3)#	2 (6.5)	0
2	0	0	3 (9.4)	0	0
3	0	1 (5.9)	0	0	2 (6.1)
No biopsy available	7 (100)	15 (88.2)	27 (84.4)	29 (93.6)	31 (93.9)
Liver/kidney health, mean (SD)					
LFC (%)	22.77 (3.1)	17.31 (5.8)	20.93 (7.2)	19.77 (6.3)	20.05 (6.7)
cT1 (ms)	866.1 (60.6)	851.8 (92.5)	903.0 (94.9)	874.5 (128.0)	924.9 (99.1)
LSM (kPa)	12.26 (6.5)	11.95 (2.9)	11.96 (4.9)	10.85 (2.0)	10.40 (2.3)
CAP (dB/m)	373.3 (33.5)	342.3 (36.8)	353.0 (29.3)	345.7 (30.1)	342.5 (24.8)
ALT (U/L)	75.6 (41.7)	41.5 (15.8)	54.0 (32.6)	55.9 (32.1)	51.6 (30.6)
AST (U/L)	48.1 (27.0)	33.5 (11.9)	34.8 (15.1)	37.1 (14.8)	33.7 (14.2)
GGT (U/L)	68.9 (45.6)	66.4 (71.3)	63.5 (43.6)	68.3 (54.7)	55.4 (25.0)
A2M (mg/dl)	221.7 (77.6)	222.3 (66.6)	218.0 (77.7)	233.4 (85.5)	204.4 (73.3)
hs-CRP (mg/L)	4.14 (2.0)	6.61 (7.4)	5.08 (5.6)	3.58 (3.2)	7.47 (8.6)
Fibrometer score	n.a.	n.a.	0.45 (0.23)	0.49 (0.24)	0.36 (0.21)
Fibrotest score	n.a.	n.a.	0.30 (0.21)	0.36 (0.25)	0.26 (0.18)
eGFR, mean (SD)	89.6 (21.7)	90.4 (22.9)	91.4 (18.7)	86.2 (16.2)	93.0 (21.7)
Lipid/metabolic, mean (SD)					
Apolipoprotein B (mg/dl)	101.6 (41.2)	90.4 (17.7)	92.1 (24.5)	97.7 (30.9)	97.6 (28.7)
Total cholesterol (mg/dl)	188.7 (66.8)	169.0 (24.1)	174.0 (39.4)	186.3 (47.4)	184.5 (43.9)
LDL-cholesterol (mg/dl)	98.2 (65.4)	94.8 (25.0)	97.8 (32.9)	104.7 (44.9)	103.1 (41.1)
HDL-cholesterol (mg/dl)	39.9 (8.3)	43.8 (6.9)	46.1 (13.0)	48.3 (13.3)	47.1 (14.2)
Triglycerides (mg/dl)	252.1 (161.7)	155.5 (94.0)	134.5 (84.0)	161.0 (78.0)	143.0 (104.0)
Uric acid (mg/dl)	6.40 (1.4)	5.61 (1.2)	5.63 (1.1)	6.09 (1.4)	5.81 (1.6)
HbA1c (%)	6.71 (1.5)	6.78 (1.2)	6.59 (1.0)	6.47 (1.3)	6.33 (1.2)

A2M, alpha 2-macroglobulin; ALP, alkaline phosphatase, ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; C4, 7-α-hydroxy-4-cholesten-3-one; CKD, chronic kidney disease; cT1, iron-corrected T1; eGFR, estimated glomerular filtration rate; HbA1c, haemoglobin A1c; hs-CRP, high-sensitivity C-reactive protein; LFC, liver fat content; LSM, liver stiffness measurement; NASH, non-alcoholic steatohepatitis; QD, once daily.
#Data from medical history biopsy report prior to screening (median –236, range -25 to -355 days), Stage 1: 1A (n = 1), 1B or 1C (n = 3). No statistical testing.

Demographic and baseline clinical characteristics were similar between the vonafexor pooled and the placebo arms and were generally representative of the typical NASH population (Table 1).

For the primary efficacy endpoint, absolute LFC (LS mean (%), [95% CI, *p* value]) decreased significantly from baseline to Week 12 with vonafexor treatment (vonafexor pooled: -4.71% [-8.47, -0.94; $p = 0.02$], placebo: 3.92%, [-2.52, 10.36; $p = 0.21$]) (Fig. S1). The difference *vs.* placebo was: -8.62%, (-16.64, -0.61; $p = 0.037$).

Secondary efficacy analyses also showed that treatment with vonafexor reduced weight, waist circumference, levels of the fibro-inflammatory imaging biomarker cT1, and improved markers of liver disease (including an initial reduction in ALT and consistent reduction in GGT; Fig. S2).

Extensive PK/PD analysis performed on Day 1 and Day 14 showed a variable vonafexor absorption phase. C_{max} was achieved between 1 and 6 h (for VONA-100BID, VONA-200QD, and VONA-400QD). On Day 1, median drug exposure (AUC_{tau}) in the treatment arms was similar (13,441 and 16,495 ng h/ml for VONA-200QD and VONA-400QD, respectively). Median C_{max} increased with dose but seemed to be less than dose-proportional (1,195, 2,220 and 3,565 ng/ml on Day 1 for VONA-100BID, VONA-200QD, and VONA-

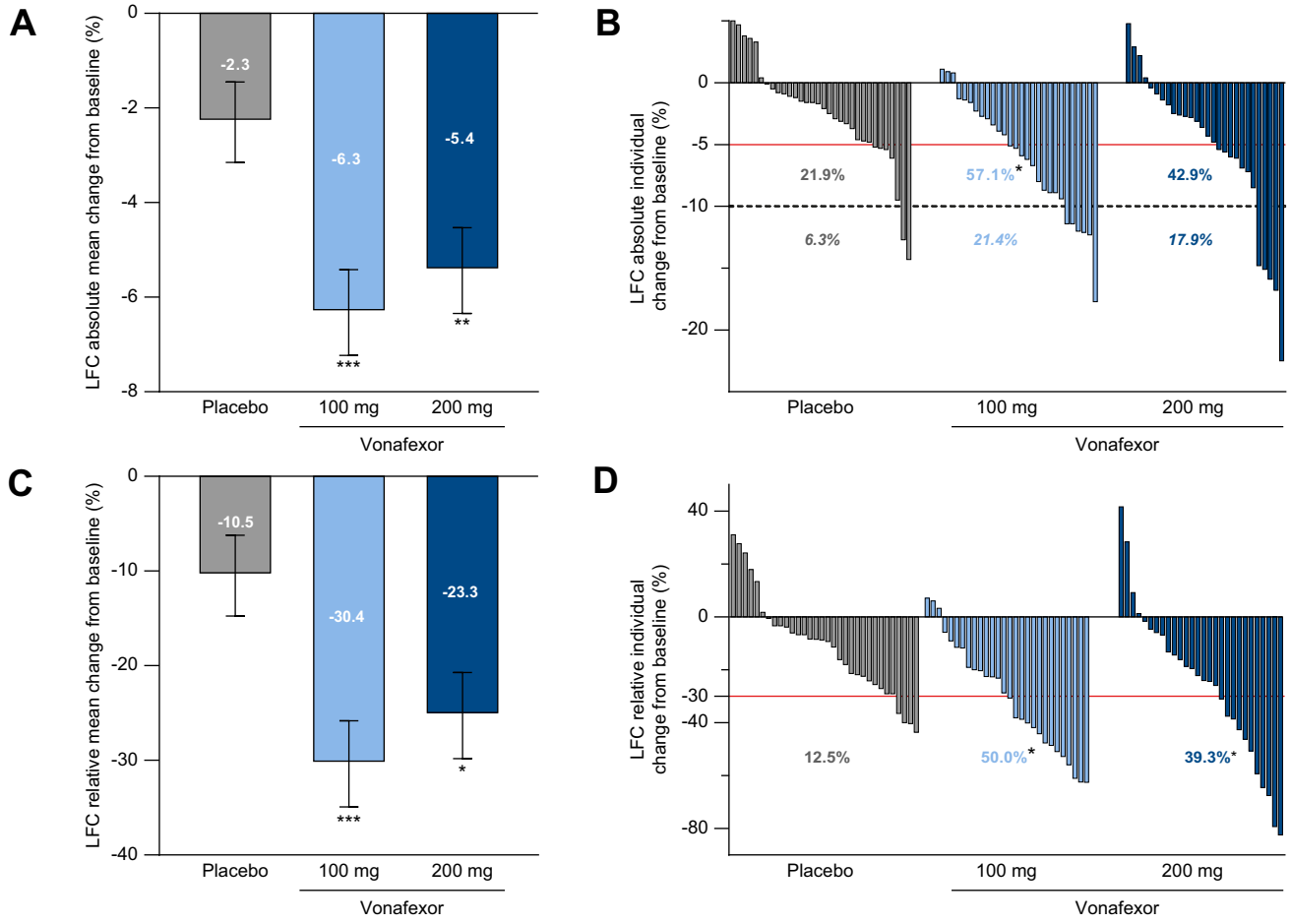


Fig. 2. Change in LFC assessed by MRI-PDFF from baseline to Week 12 (Part B). LFC LS mean (SE) change: (A) absolute, (B) absolute individual, (C) relative, (D) relative individual. Solid red line marks a 5%, dotted line a 10% absolute reduction (B), 30% relative reduction (D), % numbers show patients below cut-offs. Significance *vs.* placebo: *p <0.05, **p <0.01***, p <0.005, (ANCOVA test). LFC, liver fat content; LS, least-square; MRI-PDFF, MRI-proton density fat fraction.

400QD, respectively). C_{max} and AUC_{tau} values tended to be lower after repeated exposure on Day 14 than on Day 1.

FGF19 plasma concentrations increased and C4 levels decreased post-vonafexor dosing as expected for a nuclear FXR agonist. C4 concentrations reached a minimum between 10 and 12 h post-vonafexor dosing, before returning to levels below baseline in most patients. Minimum C4 levels were lower on Day 14 (VONA-200QD: 1.11 to 1.29 ng/ml, VONA-400QD: 0.56 to 3.73 ng/ml) after repeat vonafexor dosing than on Day 1 (VONA-200QD: 1.22 to 2.47 ng/ml, VONA-400QD: 2.46 to 10.0 ng/ml). Maximum FGF19 plasma concentrations were higher on Day 14 (VONA-100BID: 517 to 2,890 pg/ml, VONA-200QD: 3,510 to 6,740 pg/ml, and VONA-400QD: 847 to 9,640 pg/ml) than on Day 1 (VONA-100BID: 427 to 1,530 pg/ml, VONA-200QD: 678 to 1,910 pg/ml, and VONA-400QD: 290 to 4,530 pg/ml), and were achieved between 4-10 h, 6-10 h and 4-12 h before decreasing in the VONA-100BID, VONA-200QD and VONA-400QD treatment arms, respectively. For placebo, FGF19 levels were stable post-dosing, while C4 levels were lower pre-dosing.

No unexpected safety concerns with respect to liver or muscle toxicity were identified for vonafexor. The most frequent treatment-emergent adverse event (TEAE) was pruritus, reported by 16 of the 17 patients receiving vonafexor, and none of the seven patients receiving placebo. Most TEAEs were mild or moderate in severity, two severe TEAEs were reported (pruritus in the VONA-400QD arm and nephrolithiasis in the VONA-100BID arm). Grade ≥2 post-baseline aminotransferase elevations were reported for three vonafexor-treated patients in Part A, alternative aetiologies and confounding concomitant medications were identified for two. None of these events met the definition of Hy's Law for DILI. No deaths and no serious AEs were reported.

Part B – safety and efficacy of vonafexor in patients with NASH

Disposition, demographics, and baseline characteristics

In Part B, 96 of the 337 individuals screened were randomised to one of three treatment arms to receive VONA-100QD (n = 31), VONA-200QD (n = 33), or placebo QD (n = 32) (Fig. S3). All 96 patients received at least one dose of study treatment and were included in the safety and ITT population. Eighty eight of the 96 patients included in the ITT had a valid LFC measurement at both baseline and Week 12/end of treatment and were included in the mITT (n = 28 for VONA-100QD, n = 28 for VONA-200QD, and n = 32 for placebo QD).

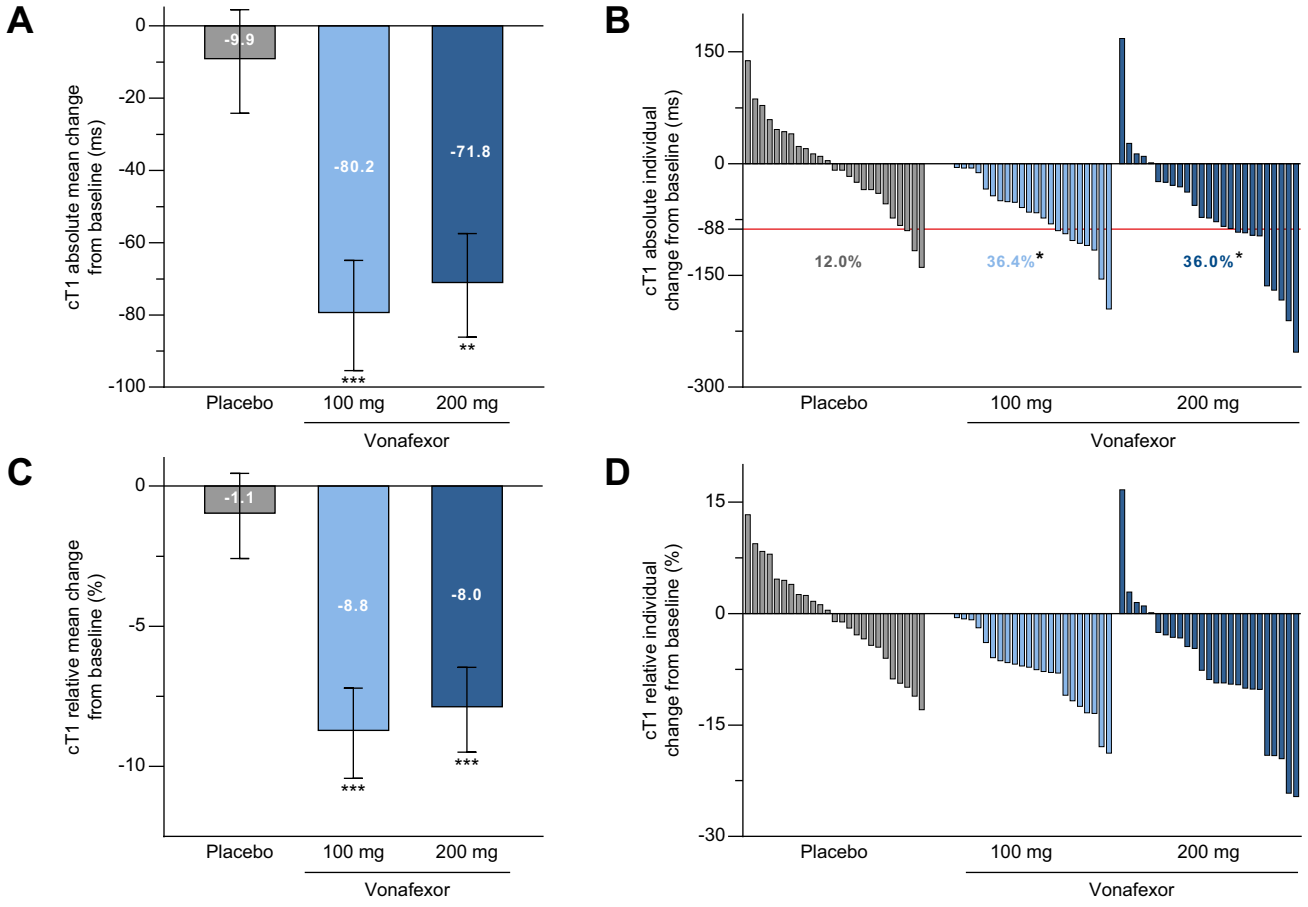


Fig. 3. Change in cT1 from baseline to Week 12 (Part B). cT1 LS mean (SE) change: (A) absolute, (B) absolute individual, (C) relative change, (D) relative individual change. Solid red line marks a -88 ms absolute reduction, % number of patients below cut-off. Significance *vs.* placebo: *p <0.05, **p <0.01, ***p <0.005, (ANCOVA test). cT1, iron-corrected T1; LS, least-square.

The demographic and baseline characteristics for the ITT population were similar across the treatment arms and were generally representative of the typical NASH population (Table 1). Similar results were reported for the mITT population.

Vonafexor efficacy

For the primary efficacy endpoint, there was a significant change in absolute LFC from baseline to Week 12 (LS mean [95% CI; p value]) across all three treatment arms (Fig. 2): -2.3%, (-4.0, -0.6; p = 0.008) for placebo, -6.3% (-8.1, -4.5; p <0.0001) for VONA-100QD and -5.4%, (-7.2, -3.6; p <0.0001) for VONA-200QD. The LFC decrease was significantly greater for the active treatment arms compared to placebo. In secondary efficacy analyses, a higher proportion of vonafexor-treated patients also achieved an absolute reduction in LFC of ≥5% and a relative reduction ≥30%.

Liver cT1 also decreased significantly from baseline to Week 12 for VONA-100QD and VONA-200QD compared to placebo (Fig. 3). A positive correlation between liver fat and cT1 changes was seen (R = 0.74, p <0.0001).

Additional secondary efficacy analyses of biomarkers of liver health and inflammation in patients who received vonafexor are presented in Table 2. Vonafexor significantly reduced serum levels of GGT at Week 12, this improvement was sustained at Week 14 (Table 2 and Fig. 4). Levels of the fibro-inflammatory marker alpha 2-macroglobulin were also markedly reduced in the vonafexor-treated arms when compared to placebo at Week 12 (Table 2 and Fig. 4).

A significant mean reduction in ALT levels from baseline to Week 12 was observed for the placebo arm and the VONA-100QD arm, but not for the VONA-200QD arm (Table 2). In a *post hoc* analysis that excluded the one patient in VONA-200QD arm who experienced a serious transaminase increase due to previously undiagnosed autoimmune hepatitis, significance was also demonstrated for VONA-200QD (Fig. 4). An additional *post hoc* responder analysis using a decrease of 17 U/L or more as a cut-off value, showed that an ALT response was achieved at Week 12 in 52% of the patients in VONA-100QD arm *vs.* 25% of patients in the placebo arm (p <0.05). There were no other clinically relevant differences found between the vonafexor and placebo arms for the other hepatic or inflammatory biomarkers assessed, except for a significant decrease in alpha 2-macroglobulin, a proteinase inhibitor known to be elevated in diabetes.

By Week 12, body weight, waist circumference, waist-to-hip ratio, and waist-to-height ratio were also significantly reduced to a greater extent in the vonafexor treatment arms compared to placebo (Fig. 4). A decrease in body weight of ≥3 kg was reported in 24.0% of patients in the VONA-100QD arm, 30.8% of patients in the VONA-200QD arm, and in 15.6% of patients in

Table 2. Changes in primary and secondary endpoints (Part B).

Parameter	Change from baseline to week 12 or week 14[a], LS mean (95% CI)			LS mean difference vonafexor *vs.* placebo (95% CI)	
	Placebo QD (n/N = 32/32)	Vonafexor 100 mg QD (n/N = 28/31)	Vonafexor 200 mg QD (n/N = 28/33)	Vonafexor 100 mg QD	Vonafexor 200 mg QD
Primary endpoint					
LFC, % absolute fat	-2.3 * (-4.0, -0.6)	-6.3 * (-8.1, -4.5)	-5.4 * (-7.2, -3.6)	-4.0 * (-6.5, -1.6)	-3.1 * (-5.6, -0.7)
Key secondary endpoints					
LFC, % relative fat	-10.5* (-19.0 -2.0)	-30.4* (-39.4, -21.3)	-25.3* (-34.3, -16.2)	-19.9* (-32.1, -7.7)	-14.8* (-27.0, -2.6)
cT1 (ms)	-9.9 (-38.5, 18.7)	-80.2* (-110.6, -49.8)	-71.8* (-100.4, -43.2)	-70.3* (-111.0, -29.5)	-61.9* (-100.8, -23.1)
Other secondary endpoints**					
Liver chemistry					
ALT (U/L)	-11.7* (-18.9, -4.6)	-16.3* (-24.1, -8.4)	-7.5 (-15.3, 0.4)	-4.5 (-15.1, 6.1)	4.3 (-6.3, 14.9)
AST (U/L)	-7.2* (-11.6, -2.8)	-5.0* (-9.8, -0.1)	0.1 (-4.7, 5.0)	2.3 (-4.2, 8.8)	7.3* (0.8, 13.9)
GGT (U/L)	-3.9 (-9.9, 2.2)	-40.6* (-47.1, -34.0)	-34.1* (-40.6, -27.7)	-36.7* (-45.6, -27.8)	-30.2* (-39.0, -21.4)
Total bilirubin (mg/dl)	-0.05* (-0.1, 0.0)	-0.1* (-0.2, 0)	-0.1* (-0.2,-0.1)	-0.05 (-0.1, 0)	-0.1* (-0.2, 0)
ALP (U/L)	1.0 (-4.9, 7.0)	19.0* (12.6, 25.5)	22.4* (15.9, 28.8)	18.0* (9.3, 26.7)	21.3* (12.6, 30.0)
Fibrosis markers					
AST/ALT ratio	-0.015 (-0.078, 0.047)	0.210* (0.142, 0.278)	0.111* (0.043, 0.178)	0.225* (0.133, 0.317)	0.126* (0.035, 0.218)
Pro-C3[a] (μg/L)	-0.7 (-3.3, 1.9)	2.6 (-0.2, 5.4)	-2.0 (-5.0, 1.0)	3.3 (-0.5, 7.1)	-1.3 (-5.2, 2.6)
Hyaluronic acid (ng/ml)[a]	9.6 (-14.6, 33.8)	8.9 (-17.8, 35.6)	-1.2 (-29.8, 27.3)	-0.7 (-36.4, 35.1)	-10.8 (-48.0, 26.4)
PIIINP[a] (ng/ml)	-0.9 (-3.1, 1.3)	2.4 (-0.1, 4.8)	-1.0 (-3.6, 1.7)	3.3 (0.0, 6.6)	-0.0 (-3.5, 3.4)
TIMP-1[a] (ng/ml)	-7.8 (-21.7, 6.2)	-4.0 (-19.2, 11.2)	-1.9 (-18.0, 14.1)	3.8 (-16.6, 24.2)	5.8 (-15.3, 27.0)
CK-18 M30[a] (U/L)	-104.3* (-192.2, -16.4)	-101.2* (-198.1, -4.3)	-16.0 (-119.0, 87.1)	3.1 (-126.8, 133.0)	88.3 (-46.2, 222.8)
CHI3L1[a] (pg/ml)	3,678 (-21,089, 28,445)	29,564* (2,991, 56,137)	5,183 (-21,936, 32,302)	25,886 (-9,945, 61,717)	1,505 (-34,863, 37,874)
ELF score	-0.02 (-0.23, 0.19)	0.11 (-0.12, 0.34)	-0.08 (-0.32, 0.17)	0.13 (-0.18,0.44)	-0.05 (-0.38, 0.27)
A2M (mg/dl)	4.3 (-3.2, 11.9)	-25.9* (-34.5, -17.4)	-18.4* (-27.3, -9.5)	-30.3* (-41.6, -18.9)	-22.8* (-34.4, -11.1)
FibroScan VCTE (kPa)	-2.0* (-3.6, -0.3)	-1.6 (-3.3, 0.0)	-2.9* (-4.6, -1.1)	0.3 (-2.0, 2.6)	-0.9 (-3.3, 1.5)
FAST score	-0.17* (-0.25, -0.09)	-0.19* (-0.29, -0.10)	-0.17* (-0.28, -0.06)	-0.02 (-0.11, 0.11)	0 (-0.13, 0.14)
CAP (dB/m)	-7.3 (-23.8, 9.15)	-26.88 (-43.8, -9.9)	-19.56 (-37.15, -1.9)	-19.56 (-42.9, 3.8)	-12.24 (-36.1, 11.6)
Fibrometer score	0 (-0.04, 0.04)	-0.06* (-0.11, -0.02)	-0.01 (-0.06, 0.04)	-0.06 (-0.13, 0.01)	-0.01 (-0.06, 0.06)
Fibrotest score[a]	0 (-0.02, 0.02)	-0.06* (-0.09, -0.03)	-0.03* (-0.06, 0)	-0.06* (-0.09, -0.02)	-0.03 (-0.07, 0)
Inflammation markers					
hs-CRP (mg/L)	-0.5 (-4.0, 2.9)	2.0 (-1.9, 5.9)	2.6 (-1.5, 6.7)	2.5 (-2.7,7.7)	3.1 (-2.4, 8.4)
Adiponectin[a] (μg/ml)	0.237 (-0.252, 0.727)	0.241 (-0.285, 0.767)	0.259 (-0.275, 0.794)	0.003 (-0.708, 0.714)	0.022 (-0.698, 0.742)
Fibronectin[a] (μg/ml)	-14.381 (-30.669, 1.907)	5.714 (-12.166, 23.595)	-12.527 (-31.428, 6.374)	20.095 (-3.906, 44.096)	1.853 (-22.832, 26.539)
IL-6[a] (pg/ml)	-0.209 (-0.486, 0.068)	-0.005 (-0.307, 0.296)	-0.122 (-0.430, 0.187)	0.204 (-0.202, 0.610)	0.087 (-0.321, 0.496)
TNF-α[a] (pg/ml)	-0.261* (-0.455, -0.066)	0.025 (-0.186, 0.237)	-0.075 (-0.296, 0.145)	0.286* (0.002, 0.570)	0.185 (-0.105, 0.476)
Lipid panel					
Apolipoprotein B (mg/dl)	-7.1 (-15.1, 0.8)	19.7* (11.1, 28.4)	20.0* (11.4, 28.5)	26.8* (15.1, 38.5)	27.1* (15.4, 38.7)
Total cholesterol (mg/dl)	-10.1 (-22.7, 2.5)	18.7* (5.0, 32.4)	19.3* (5.8, 32.8)	28.8* (10.3, 47.3)	29.4* (10.9, 47.8)
LDL-cholesterol (mg/dl)	-7.9 (-18.9, 3.1)	20.4* (8.4, 32.4)	22.2* (10.2, 34.2)	28.3* (12.1, 44.6)	30.1* (13.9, 46.4)
HDL-cholesterol (mg/dl)	0.3 (-2.5, 3.1)	-8.4* (-11.5, -5.3)	-5.8* (-8.8, -2.8)	-8.7* (-12.8, -4.5)	-6.1* (-10.2, -2.0)
Triglycerides[b] (mg/dl)	-2.0 (54.0)	24.0* (57.0)	23.0 (71.0)	31.0* (6.0, 60.0)	27.5 (-5.0, 53.0)
Metabolic panel					
HOMA index	6.1* (1.9, 10.3)	-0.6 (-5.3, 4.2)	-0.1 (-5.0, 4.8)	-6.8 (-14.7, 1.1)	-5.9 (-13.9, 2.1)
HbA1c[a] (%)	-0.02 (-0.38, 0.34)	0.29 (-0.10, 0.68)	0.15 (-0.24, 0.54)	0.31 (-0.22, 0.84)	0.17 (-0.36, 0.70)
Kidney panel					
eGFR (ml/min/1.73m^2)	-2.7 (-6.6, 1.2)	6.2* (1.9, 10.2)	3.2 (-1.2, 7.7)	8.9* (3.1, 14.7)	5.9* (0.1, 11.8)
BUN (mg/dl)	-0.1 (-1.1, 0.9)	0.3 (-0.8, 1.4)	-0.1 (-1.2, 1.1)	0.4 (-1.1, 1.9)	0.0 (-1.6, 1.5)
Uric acid (mmol/L)	-0.23 (-0.47, 0.01)	-0.25 (-0.52, 0.02)	-0.43* (-0.71, -0.15)	-0.02 (-0.37, 0.34)	-0.2 (-0.57, 0.17)

A2M, alpha 2-macroglobulin; ALP, alkaline phosphatase, ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CHI3L1, chitinase-3-like protein 1; CK-18, cytokeratin-18; cT1, iron-corrected T1; eGFR, estimated glomerular filtration rate; ELF, enhanced liver fibrosis; GGT, gamma-glutamyltransferase; HbA1c, haemoglobin A1c; HOMA, homeostatic model assessment; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; LFC, liver fat content; PIIINP, procollagen type III; Pro C3, released N-terminal pro-peptide of type III collagen; TIMP-1, tissue inhibitor of metalloproteinases-1; TNF-α, tumour necrosis factor-alpha.

*p <0.05 (LFC and cT1: ANCOVA test. Triglycerides: Wilcoxon. All other endpoints: mixed model for repeated measures testing). Negative values for LS mean difference, 95% CI, denote improvement *vs*. the placebo arm (except for eGFR and cholesterol). n: number of patients with a value at baseline and Week 12 or Week 14; N, intent-to-treat population.

** For all other secondary endpoints, n = N.

[a]Values represent change from baseline to Week 14.

[b]Median (interquartile range) and location shift (95% CI, Wilcoxon-test).

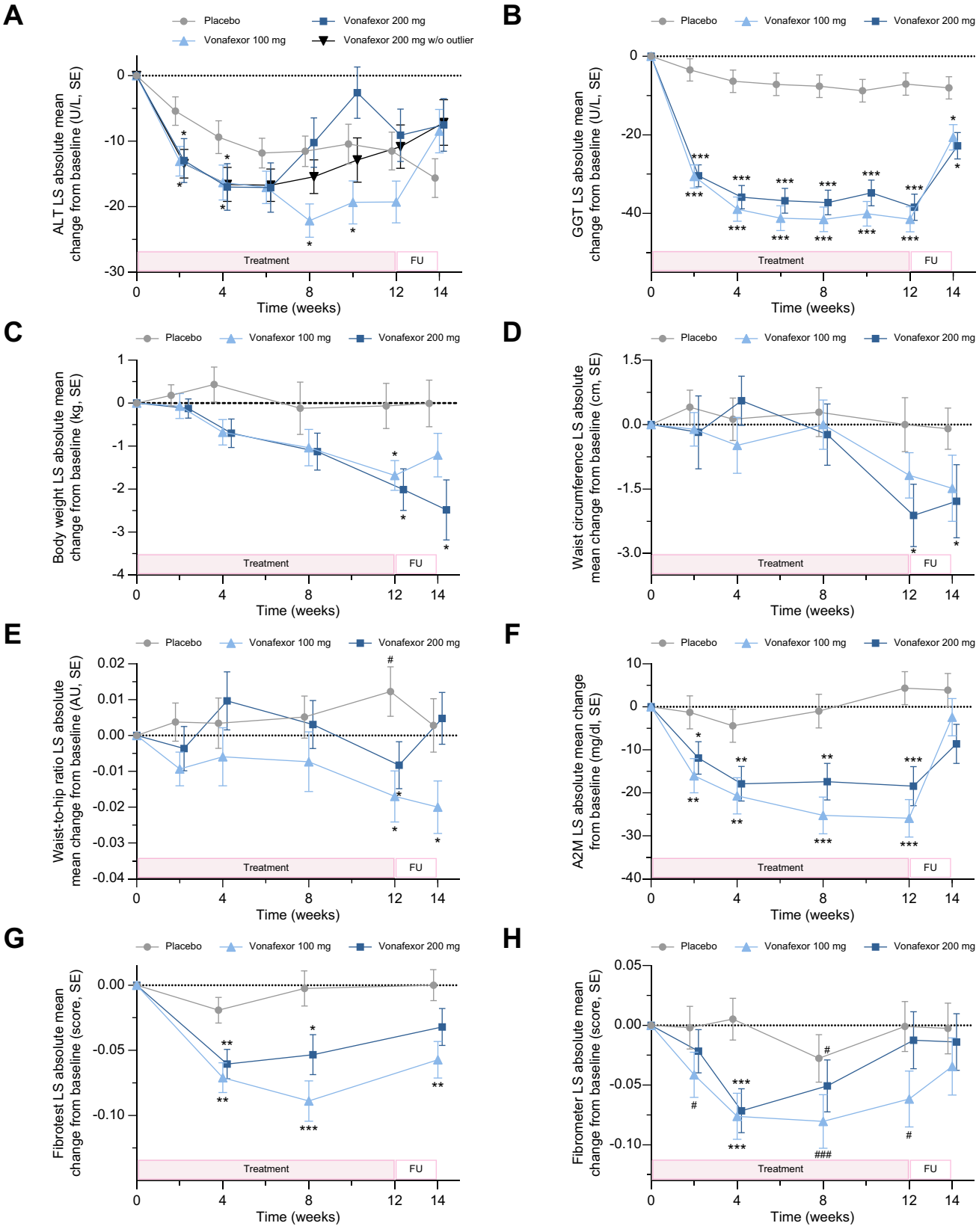


Fig. 4. Secondary outcomes (Part B). LS absolute mean (SE) change: (A) excluding patient with autoimmune hepatitis, (B) GGT, (C) body weight, (D) waist circumference, (E) waist-to-hip-ratio, (F) A2M, (G) Fibrotest, (H) Fibrometer. Significance *vs.* placebo: $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.005 *vs.* baseline: $^{\#}p$ <0.05, $^{\#\#\#}p$ <0.005, (MMRM testing). A2M, alpha 2-macroglobulin; AU, arbitrary unit; GGT, γ-glutamyltransferase; LS, least-square.

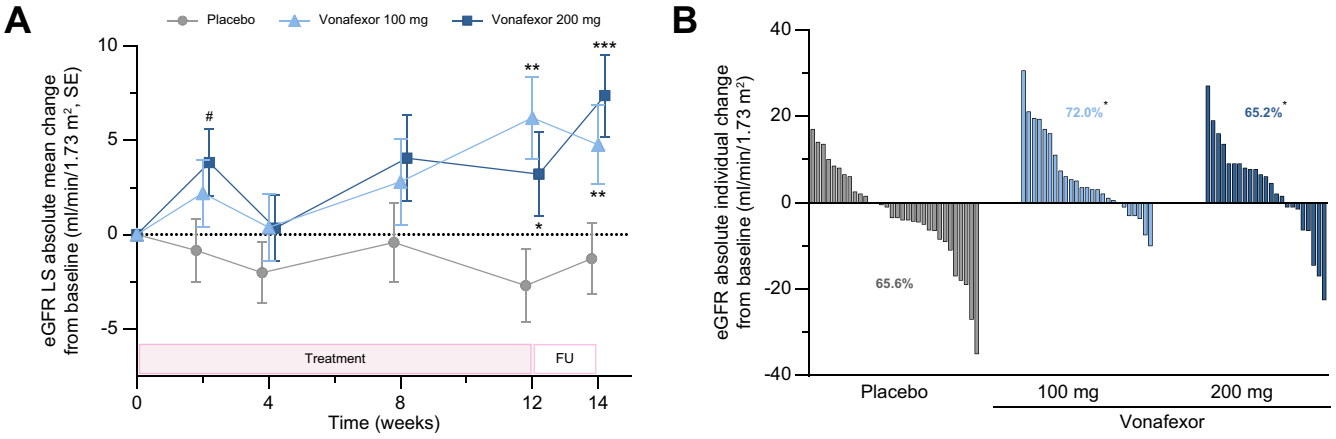


Fig. 5. Change in eGFR (Part B). LS mean (SE) change: (A) absolute, (B) absolute individual, % number of patients with positive change. Significance *vs.* placebo: **p* <0.05, ***p* <0.01, ****p* <0.005, (MMRM testing). eGFR, estimated glomerular filtration rate; LS, least-square.

the placebo arm. Changes in liver stiffness, as assessed by FibroScan VCTE, were not significantly different from baseline to Week 12 for the VONA-100QD arm (Table 2). However, *post hoc* Fibrotest analyses showed a significant improvement from baseline to Week 14 for the VONA-100QD and VONA-200QD arms (Table 2).

Changes in glomerular filtration rate

eGFR (LS mean, 95% CI, [ml/min/1.73m^2]) significantly increased in both vonafexor treatment arms at Week 12 and Week 14: 6.2 (1.9, 10.2; $p = 0.006$) and 4.8 (0.6, 9.0; $p = 0.03$) for VONA-100QD, and 3.2 (-1.2, 7.7; $p = 0.15$) and 7.4 (3.0, 11.7; $p = 0.001$) for VONA-200QD. In contrast, mean eGFR decreased by -2.7 (-6.6, 1.2; $p = 0.17$) and -1.3 (-5.0, 2.5; $p = 0.5$) at Week 12 and Week 14, respectively, in the placebo arm (Fig. 5). The results were qualitatively unchanged when eGFR was calculated using the current CKD-Epidemiology Collaboration (EPI; 2021) formula (data not shown).[23,24] Significantly more patients in the vonafexor arms experienced an increase in eGFR than in the placebo arm (Fig. 5B).

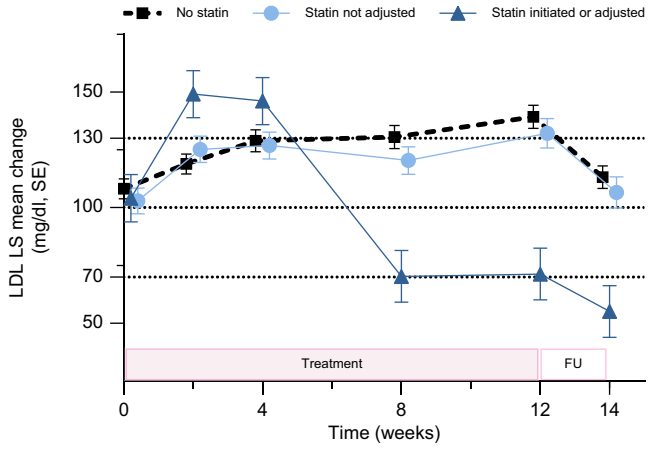


Fig. 6. LDL-cholesterol increases managed by statin introduction or adjustment in vonafexor-treated patients (Part B). No statin: patients without statin at any timepoint, treated with VONA-100QD (n = 18) or VONA-200QD (n = 19). Statin not adjusted: patients on baseline stable statin without dose changes, on VONA-100QD (n = 9) or VONA-200QD (n = 12). Statin initiated or adjusted: patients with baseline statin dose increased (n = 2) or statin initiation (n = 4) while on VONA-100QD (n = 4) or VONA-200QD (n = 2) (Descriptive statistics, no hypothesis testing). FU, follow-up; VONA-100QD, vonafexor-100 mg once daily; VONA-200QD, vonafexor 200 mg once daily.

Safety and tolerability

The overall incidence of TEAEs was higher for the vonafexor arms compared to placebo. The most frequent TEAEs in the vonafexor arms were pruritus and pruritus generalised, which occurred in 19 (61.3%) and 3 (9.7%) of the 31 patients in the VONA-100QD arm, 17 (51.5%) and 6 (18.2%) of the 33 patients in the VONA-200QD arm, and in 2 (6.3%) and 2 (6.3%) of the 32 patients in the placebo arm. Most TEAEs were mild to moderate in severity (VONA-100QD: 61.3%, VONA-200QD: 75.8%, and placebo: 65.6%).

Two patients reported an AESI of aminotransferase elevation considered by the investigator to be unrelated to the study drug (VONA-100QD: n = 1, VONA-200QD: n = 1). Neither of these events met the definition of Hy's Law, the DILI stopping criteria, nor the protocol-defined stopping rules for decreased liver function. Four patients experienced muscle-related AESIs (VONA-100QD: n = 1, placebo n = 3), none of these events were considered as related to the study drug or statin use.

Severe TEAEs occurred in 11 patients (11.5%; VONA-100QD: n = 5, VONA-200QD: n = 5, placebo: n = 1). Serious TEAEs were reported by one patient in the placebo arm (vertigo), one patient in the VONA-100QD arm (respiratory failure and angina unstable), and two patients in the VONA-200QD arm (coronavirus infection and transaminases increased). None were considered to be drug-related.

Some vital sign, electrocardiogram, and physical examination values fell outside of the normal limits, but no clinically relevant trends or results were noted for any of the parameters tested. Clinical laboratory analysis also revealed a change from baseline for some lipid panel parameters with vonafexor treatment; these included changes in apolipoprotein B, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides (Table 2). Most differences were not considered to be clinically relevant, however, a clinically relevant increase in LDL (>130 mg/dl) was observed in six vonafexor-treated patients. After statin treatment was initiated in four of these

patients and existing statin doses were increased in the other two patients, LDL levels subsequently decreased to within the normal range (Fig. 6).

Study discontinuation due to a TEAE occurred in four patients in the VONA-100QD arm (2 pruritus, 1 pruritus generalised and 1 rash with pruritus) and eight patients in the VONA-200QD arm (2 pruritus, 2 pruritus generalised, 1 abdominal pain upper, 1 transaminase increased, 1 depression, and 1 malaise). There were no deaths reported in Part B.

Discussion

This phase IIa, randomised, placebo-controlled trial demonstrated that vonafexor, a second-generation, non-bile acid FXR agonist, strongly reduces LFC in patients with NASH after only 12 weeks of treatment. This beneficial effect is corroborated by an improvement in hepatic enzymes (including ALT and GGT), and in imaging biomarkers of fibro-inflammation, including corrected T1, an MRI parameter associated with hepatic outcomes.[25] The reduction in body weight and visceral obesity (waist circumference) seen with vonafexor treatment is also a favourable outcome, as most patients with NASH are overweight or obese. Weight loss with vonafexor was higher than that observed after 72 weeks of treatment with obeticholic acid (OCA), a first-generation FXR agonist, and similar to that reported with tropifexor (52 weeks), another second-generation FXR agonist.[13,26] Finally, vonafexor also significantly improved eGFR when compared to placebo, suggesting that it may improve kidney function; a significant finding in patients with NASH who often manifest multiple risk factors for kidney injury and are at high risk of developing progressive CKD.

Although the magnitude of weight loss was higher than expected, based on longer trials with OCA[13] or cilofexor,[27] this cannot account for the loss of liver fat observed with vonafexor. Importantly, half of the VONA-100QD arm achieved a 30% relative reduction in liver fat from baseline; a level associated with histological improvement in the NAFLD activity score, resolution of steatohepatitis, and a reduction of liver fibrosis, defining MRI-PDFF responders in clinical trials.[28,29] This was a fourfold increase over the placebo arm, which is higher than reported for tropifexor after 48 weeks of treatment (2.4 fold for the 200 μg arm over placebo), and EDP-305 after 12 weeks of treatment (1.8 fold for the 2.5 mg arm over placebo).[30,31] In addition, 57% of patients in the VONA-100QD arm achieved a ≥5% absolute reduction in LFC, a level associated with histological improvement in patients with advanced disease at baseline.[32–35] While the relationship between LFC reduction and histological resolution of steatohepatitis may not apply to all drugs,[36] studies suggest that this may be the case for drugs with pleiotropic effects, such as FXR agonists, or drugs with a strong defattening effect (*e.g.*, resmetirom, or aldafermin).[33,37] Mechanistically, the reduction in hepatic triacylglycerols with FXR agonists is related to inhibition of both hepatic lipogenesis and intestinal absorption of lipids.[38] The former involves a SHP-SREBP1c-independent inhibition of key lipogenic enzymes such as stearoyl CoA desaturase, Lpin1, and DGAT2. The latter is mediated by the deficit in bile acid synthesis induced by direct hepatic FXR agonism.[38]

While this short proof-of-principle trial was not designed to assess liver histological endpoints, it is noteworthy that cT1, a quantifiable marker of fibro-inflammation in patients with steatohepatitis, was significantly reduced with vonafexor treatment.[39–41] The magnitude of the reduction seen in the VONA-100QD arm was similar to that associated with a 2-point improvement in NAFLD activity score (88 ms), as reported in non-interventional follow-up trials.[40] How this relates to histological improvement remains to be determined, however, interim results from a subset of the REGENERATE trial showed an association between the histological response to OCA and a reduction in cT1.[42]

The positive outcomes of vonafexor on radiological parameters and body weight were further supported by improvements in biomarkers of liver injury and inflammation, including ALT and GGT.[43,44] A reduction in ALT of ≥17 U/L (observed here in over half of patients in the VONA-100QD arm) was associated with histological improvement in the OCA FLINT study.[35] LIVIFY was a short-term 12-week study, and the timing and rate of the LFC response can vary depending on the mode of action.[34] Recently Huang *et al.* showed that a combined MRI-PDFF and ALT response of ≥30% and ≥17 U/L, respectively, was associated with higher odds of histologic response than either response alone.[45] Interestingly, 19% of vonafexor-treated patients with NASH showed a combined response (*vs*. 11% in the Huang paper) which suggests a likely histological response in these patients. Future studies should determine if longer durations of vonafexor therapy will result in enhanced reduction of GGT and ALT, or in a higher proportion of responders.

The primary objective of NASH therapies is to prevent progression to cirrhosis. However, NASH is part of a multisystem syndrome of metabolic dysregulation and inflammation involving the kidney and cardiovascular systems. In the current study, vonafexor increased eGFR, suggesting renal benefits of vonafexor treatment in NASH. Additional research is needed to interpret the underlying mechanisms of this potential improvement. Theoretically, the weight loss induced by vonafexor could have reduced creatinine production and thereby raised eGFR without altering kidney function. It will therefore be important to first investigate whether the increase in eGFR represents an actual increase in GFR as measured by a direct gold standard technique such as iohexol clearance.[46,47] The finding that vonafexor simultaneously decreased serum uric acid levels suggests a true increase in GFR. Second, it will be important to determine whether a true increase in GFR reflects acute and rapidly reversible hemodynamic effects of vonafexor, or long-term benefits on kidney structure and function. In support of beneficial effects on the kidney, FXR is highly expressed in healthy kidneys and is downregulated in human and animal CKD;[48,49] in contrast, FXR activation in a variety of animal models prevented loss of kidney function and inhibited interstitial fibrosis, akin to the anti-fibrotic effects of FXR agonism in the liver.[50–52] Quantitative measurements of albuminuria, which could have helped tease apart the contribution of hemodynamic *vs.* other mechanisms, were unfortunately not available. For example, increased albuminuria in the setting of increased eGFR would suggest induction of glomerular hyperfiltration, which is likely to foreshadow undesirable long-term effects on the kidney, whereas decreased or unchanged albuminuria in the setting of increased eGFR would suggest a potential long-term benefit in preventing CKD and slowing progression. Studies of long-term vonafexor exposure with longer observation of kidney function after withdrawal of vonafexor are needed.

Patients with NAFLD also display atherogenic dyslipidaemia.[53] FXR agonism regulates lipid and lipoprotein metabolism through multiple mechanisms,[54] including enhanced cholesteryl ester transfer protein activity, increased reverse cholesterol transport and absorption, and reduced LDL-receptor expression in hepatocytes. However, different subpopulations of lipid particles confer different atherogenic risks, and results can differ depending on the duration of exposure.[55] Treatment with the parent compound of the FXR agonist class, OCA, induces complex modifications including increases in the more atherogenic small dense LDL particles, and an increase in small VLDL particles that are less atherogenic.[15] While an increase in LDL with OCA is very common (85% of treated patients in the first month),[55] and can reach 35% from baseline values,[13] it is entirely reversible upon discontinuation of the drug,[15] or with statin treatment.[55] Although some of the non-biliary FXR agonists have shown a lower magnitude of LDL increase than OCA,[27,56] this may reflect a less potent PD response, as measured by C4 reduction or FGF19 increase.[27] The current trial of vonafexor documented changes in lipid profiles similar in magnitude to those induced by OCA.[13,15] Studies in rodents with humanised livers have shown that non-steroidal FXR agonists induced lipid changes similar to OCA,[57] which favours the view that LDL increases are a class effect of FXR agonists. The longer term clinical relevance of these lipid changes remains unknown. Although data so far is limited, there is no indication that OCA-induced lipid changes result in a substantial modification of cardiovascular risk scores,[15,35] or an increase in clinical cardiovascular AEs.[13] Conversely, studies of FXR agonists in murine models have shown reduced atherosclerotic plaque formation.[58–60] Hence, the clinical significance of lipid changes induced by vonafexor needs to be determined in long-term studies.

Another drawback commonly associated with FXR agonism is pruritus, also confirmed with vonafexor. Pruritus and pruritus generalised were the most frequently reported TEAEs in the vonafexor arms, although most were mild to moderate in severity, resulting in discontinuation in fewer than 10% and 5% of patients, respectively. Although the mechanisms for pruritus occurrence are unknown, other studies with different FXR agonists have shown that it is clearly a dose-dependent class effect.[31,56] Baseline pruritus has been documented in patients with NASH and could be a predictor of the incidence and severity of on-treatment pruritus.[61] Nonetheless, patient-reported outcomes were improved in histological responders to OCA despite baseline or treatment-induced pruritus.[61,62] Dose adjustment may therefore be important to derive maximal histological benefit while reducing the incidence and severity of pruritus.

The Part A safety and PK indicated that it was safe to proceed with Part B. However, VONA-400QD had the highest score for pruritus with no additional benefit seen on efficacy. To optimise the risk/benefit balance this dose was dropped and all vonafexor-treated patients in Part B started on VONA-100QD, with the dose increased to 200 mg after 2 weeks in those randomised to VONA-200QD. However, this run-in period did not have an impact on pruritus. The PK profile of vonafexor, which associates a short half-life of 2-4 h and a decrease in exposure after a few days, showed improved daily coverage, which can be attributed to liver-enriched concentrations (data from *in vivo* quantitative whole-body autoradiography not shown). One possible explanation for the absence of a clear dose effect on liver fat reduction, ALT decline and other hepatic endpoints is that doses above 100 mg QD achieve a saturation of the hepatic nuclear FXR transcriptional response, with no additional downstream effector activity, leading to no additional effect size on liver efficacy endpoints. Interestingly, no dose effect was observed for either C4 or FGF-19 PD markers. In contrast, the higher incidence of pruritus observed with the doubling of the dose of vonafexor indicates that pruritus may be dependent on poorly identified peripheral mechanisms, and therefore more directly related to plasma PK exposure. The presumed PK liver enrichment translated into a strong modulation of target genes, including a >90% reduction in C4 and a 10-to 30-fold increase in FGF19. These PD changes are comparable with, or higher than, those reported with other potent FXR agonists, yet they do not translate into unknown side effects or unexpected tolerability issues. Importantly, glycaemic parameters and surrogate measures of insulin resistance were unaffected by vonafexor, thereby confirming safe use of this compound in patients with T2DM or pre-diabetes.

In conclusion, the LIVIFY trial shows that vonafexor induces potent liver fat reduction, improvement in liver enzymes and in imaging biomarkers of fibrotic steatohepatitis, in addition to a potential improvement in kidney function. These results deserve to be further investigated in longer and larger trials.

Affiliations

[1]Sorbonne Université, ICAN, Hospital Pitié-Salpêtrière, INSERM UMRS 1138 CRC, Paris, France; [2]Pinnacle Clinical Research, San Antonio, TX, USA; [3]Department of Liver Disease, Limoges University Hospital Center, U1248, INSERM, F87000, Limoges, France; [4]Service d'Hépatologie, Hopital Rangueil CHU, Toulouse, France; [5]Université Paul Sabatier, Toulouse, France; [6]The Texas Liver Institute, San Antonio, TX, USA; [7]University of Texas Health, San Antonio, TX, USA; [8]Mayo Clinic, Rochester, MN, USA; [9]Arizona Liver Health, Chandler, AZ, USA; [10]Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerp, Belgium; [11]ENYO Pharma SA, Lyon, France; [12]Division of Nephrology, Department of Medicine and Duke Clinical Research Institute, Durham, NC, USA; [13]Institute of Liver Disease and Metabolic Health, Interim Chair, Division of Gastroenterology, Virginia Commonwealth University, VA, USA.

Abbreviations

AE, adverse events; AESI, adverse events of special interest; ALT, alanine aminotransferase; ANCOVA, analysis of covariance; AST, aspartate aminotransferase; C4, 7-α-hydroxy-4-cholesten-3-one; CKD, chronic kidney disease; cT1, iron-corrected T1; DILI, drug-induced Liver Injury; eGFR, estimated glomerular filtration rate; FGF19, fibroblast growth factor 19; FXR, farnesoid X receptor; GGT, gamma-glutamyltransferase; ITT, intent-to-treat; LFC, liver fat content; LS, least-square; LSM, liver stiffness measure; PDFF, proton density fat fraction; mITT, modified intent-to-treat; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OCA, obeticholic acid; PD, pharmacodynamics; PK, pharmacokinetics; QD, once daily; TEAE, treatment-emergent AE; T2DM, type 2 diabetes mellitus; ULN, upper limit of normal; VCTE, vibration-controlled tissue elastography; VONA-100BID, vonafexor 100 mg twice daily; VONA-100QD, vonafexor-100 mg once daily; VONA-200QD, vonafexor 200 mg once daily; VONA-400QD, vonafexor-400 mg once daily.

Financial support

The study was supported and funded by ENYO Pharma, SA., Lyon, France.

Conflict of interest

VR has received consulting fees from: Boehringer-Ingelheim, ENYO Pharma, Novo-Nordisk, Galmed, Terns, Theratechnologies, Bristol-Myers-Squibb, Genfit,

Madrigal, and NGM Bio. SH has attended advisory boards or acted as a consultant for: Akero Therapeutics, Alentis Therapeutics, Alimentiv, Altimmune Inc., Arrowhead Pharmaceuticals, Axcella Health, Chronwell, Corcept Therapeutics, Echosens North America, ENYO Pharma, Galectin Therapeutics, Genfit, Gilead Sciences, Hepion Pharmaceuticals, Hightide Therapeutics, Histoindex, Intercept, Madrigal Pharmaceuticals, Medpace, NGM Biopharmaceuticals, Northsea Therapeutics, Novartis, Novo Nordisk, PathAI, Perspectum, Poxel, Sagimet Biosciences, Sonic Incytes, Terns, Theratechnologies, and Viking; SH has stock options in: Akero, Chronwell, Cirius, Galectin, Genfit, Hepion, HistoIndex, Metacrine, NGM Bio, and Northsea Therapeutics; SH has also received grant/research support from: Akero, Axcella, BMS, Cirius, CiVi Biopharma, Conatus, Cymabay, ENYO Pharma, Galectin, Genentech, Genfit, Gilead, Hepion, Hightide, Intercept, Madrigal, Metacrine, NGM Bio, Novartis, Novo Nordisk, Northsea Therapeutics, Pfizer, Sagimet, Viking, and 89 Bio. VLR had a contract with/grant paid to her institution by: Genfit (Resolve it study), Allergan (Aurora study), Madrigal (MGL-3196-11 study), Lilly (Synergy NASH study), Gilead (Stellar study), ENYO Pharma (LIVIFY Study). CB had a contract with/a grant paid to his institution by: ENYO Pharma (LIVIFY Study) and Gilead; CB received consulting fees from Abbvie and payment or honoraria for lectures, presentations, manuscript writing or educational events from: Gore. CB also received support to attend meetings and/or a travel from: Gilead and Abbvie. EL was a researcher for: 89Bio Inc., AbbVie, Akero Therapeutics, Allergan, Alnylam Pharmaceuticals Inc., Amgen, Ascelia Pharma, Assemblybio, Astrazeneca, Axcella Health, Biocryst Pharmaceuticals, Bird Rock Bio Inc., Boehringer Ingelheim, Bristol-Myers Squibb, Conatus Pharmaceuticals, Cymabay Therapeutics, CytoDyn, DSM, Durect Corporation, Eli Lilly and Company, Enanta Pharmaceuticals, ENYO Pharma, Exalenz Bioscience, Galectin Therapeutics, Galmed Pharmaceuticals, Genfit, Genentech, Gilead Sciences, GlaxoSmithKline, Hanmi Pharmaceuticals, Hightide Biopharma, Intercept Pharmaceuticals, Inventiva, Janssen Pharmaceuticals, Laboratory for Advanced Medicine, Loxo Oncology, Madrigal Pharmaceuticals, Merck & Co., Metacrine, NGM Biopharmaceuticals Inc., Northsea Therapeutics, Novartis, Novo Nordisk Inc., Pfizer, Poxel Co., Roche, Sagimet Biosciences, Synlogic Therapeutics, Terns Pharmaceuticals, Viking Therapeutics, and Zydus Pharmaceuticals. MA: has attended advisory boards or acted as a consultant for: Bristol-Myers Squibb, Hanmi Pharmaceuticals, Madrigal Pharmaceuticals, NGM Bio, Novartis, Novo Nordisk, Soni Incytes, Theratechnologies; MA has received grant/research support from: Allergan, Boehringer-Ingelheim, Bristol-Myers Squibb, Conatus, Celgene, ENYO Pharma, Galectin, Genentech, Genfit, Gilead, Intercept, Hanmi, Madrigal, NGM Bio, Novartis, Novo Nordisk, Viking, and 89 Bio. NA: had a contract with/received a grant from: 89Bio, Akero, AbbVie/Allergan, Better Therapeutics, Boehringer Ingelheim, Bristol-Myers Squibb, Corcept, Galectin, Genentech, Genfit, Gilead, Hepagene, Healio, Intercept, Inventiva, Ionis, Madrigal, Merck, NGM, Noom, NorthSea, Novo Nordisk, Perspectum, Pfizer, Poxel, Viking, and Zydus; NA consulted for: AbbVie/Allergan, Echosens, Gilead, Intercept, Madrigal, Novo Nordisk, Perspectum, Pfizer, and Zydus; NA received payment or honoraria for lectures, presentations, manuscript writing or educational events from: AbbVie/Allergan, Alexion, Echosens, Eisai, Exelixis, Gilead, Intercept, Perspectum, Salix, and Theratechnologies. NA also has stock or stock options with: AGED diagnostics. SF holds a senior clinical investigator fellowship from the Research Foundation Flanders (FWO) (1802154 N). His institution has received grants from Astellas, Falk Pharma, Genfit, Gilead Sciences, GlympsBio, Janssens Pharmaceutica, Inventiva, Merck Sharp & Dome, Pfizer, Roche. SF has acted as consultant for Abbvie, Actelion, Aelin Therapeutics, AgomAb, Aligos Therapeutics, Allergan, Astellas, Astra Zeneca, Bayer, Boehringer Ingelheim, Bristoll-Meyers Squibb, CSL Behring, Coherus, Echosens, Eisai, ENYO Pharma, Galapagos, Galmed, Genetech, Genfit, Gilead Sciences, Intercept, Inventiva, Janssens Pharmaceutica, Julius Clinical, Madrigal, Medimmune, Merck Sharp & Dome, NGM Bio, Novartis, Novo Nordisk, Promethera, Roche. SF has been a lecturer for Abbvie, Allergan, Bayer, Eisai, Genfit, Gilead Sciences, Janssens Cilag, Intercept, Inventiva, Merck Sharp & Dome, Novo Nordisk, Promethera. HG is an ENYO Pharma employee. RD is an ENYO Pharma employee. HC was an ENYO Pharma employee. MW served as a consultant for Amgen, Bayer, Jnana, Reata, and Pharmacosmos, as a member of the Scientific Advisory Board for Unicycive and Walden, and as a member of the Board of Directors of Akebia. AS has stock options in Genfit, Akarna, Tiziana, Indalo, Durect Inversago and Galmed. He has served as a consultant to ENYO Pharma, Astra Zeneca, Nitto Denko, Conatus, Nimbus, Salix, Tobira, Takeda, Jannsen, Gilead, Terns, Birdrock, Merck, Valeant, Boehringer-Ingelheim, Bristol Myers Squibb, Eli Lilly, Hemoshear, Novartis, Novo Nordisk, Pfizer, Exhalenz, Alnylam, Regeneron, Genentech, Sorrozen, Poxel, 89Bio, Path AI, Histoindex and Genfit. His institution has received grant support from Gilead, Salix, Tobira, Bristol Myers, Shire, Intercept, Merck, Astra Zeneca, Malinckrodt, Cumberland and Novartis. AS also receives royalties from Elsevier and UptoDate. JV is an ENYO Pharma employee. PS is an ENYO Pharma consultant.

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

Authors' contributions

The study was designed in conjunction with the authors. ENYO Pharma was involved in study design, data collection, data analysis, data interpretation, and writing of the report. All authors had full access to all the data in the study, participated in drafting and editing the manuscript and were responsible for the decision to submit for publication.

Data availability statement

ENYO Pharma shares anonymised individual patient data upon request or as required by law and/or regulation with qualified external researchers. Approval of such requests is at ENYO Pharma'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.

Committee(s) approving the study protocol

- **US:** www.sterlingirb.com e-mail info@sterlingirb.com.
- **Belgium:** MEDICAL ETHICS COMMITTEE UZA, Wilrijkstraat 10/2650 EdegemParking via Drie Eikenstraat 655 www.uza.be/BE0874.619.603.
- **France:** SOUTHWEST AND OVERSEAS TERRITORIES III ETHICS COMMITTEE Department of Medical Pharmacology-Groupe Hospitalier Pellegrin-Bât. 1APlace Amélie Raba-Léon -33076 Bordeaux France.
- **UK:** NHS HRA London -West London & GTAC Research Ethics CommitteeThe Old ChapelRoyal Standard PlaceNottinghamNG1 6FS.

Acknowledgments

The authors acknowledge the support of the following clinical investigators: Anja Geerts (University Hospital Gent), Christophe Van Steenkiste (General Hospital Maria Middelares), Christophe Moreno (Université Libre de Bruxelles (ULB) - Hopital Erasme), Victor De Ledinghen (CHU Bordeaux - Hôpital Haut-Lévêque), Guru Aithal (Nottingham NHS Treatment Centre), Michael Allison (Cambridge University - Addenbrooke's Hospital), William Alazawi (The Royal London Hospital), Roger McCorry (Royal Victoria Hospital), Kosh Agarwal (King's College Hospital), William Bowman (Sensible Healthcare, LLC), Jeffrey Williams (Summit Clinical Research, LLC), Gary Reiss (Tandem Clinical Research), Reem Ghalib (Texas Clinical Research Institute), John Lentz III (Georgia Clinical Research, LLC), Samir Arora (Aventiv Research, Inc.), Brian Borg (Southern Therapy and Advanced Research LLC), Christopher Christensen (Texas Digestive Disease Consultants), Bradley Freilich (Kansas City Research Institute), Nadege Gunn (Pinnacle Clinical Research - Austin, TX), Marcel Twahirwa (Doctor's Hospital at Renaissance (DHR) - Transplant Institute and Liver Specialty Center), Alexander White (Progressive Medical Research), Johnny White (Bioclinica Research), Blake Jones (Innovative Clinical Research – Lafayette), Paul Thuluvath (Mercy Medical Center - Baltimore, Maryland), Robert Murphy (Arkansas Gastroenterology - North Little Rock), Ann Moore (Arizona Liver Health – Glendale), Joseph Galati (American Research Corporation), Stanley Hsin-Wei Hsia (National Research Institute - Huntington Park), Grisell Ortiz-Lasanta (Fundacion de Investigacion (FDI), Malisa Agard (ClinCloud LLC), Ravi Ravinuthala (Consultants for Clinical Research – Cincinnati), Guy Neff (Covenant Research, LLC), Lincoln Hernandez (Tandem Clinical Research GI- New York).

The authors also acknowledge the support of Maud Fischer, Gregory Hood and all the Medpace, Inc. team for the overall management of the clinical trial, Charlotte Erpicum, Henrike Puchta and the rest of Perspectum Diagnostics, Ltd. team for the imaging management and analyses, Sophie Jeannin, Gail Hinkson, Genevieve Long and the rest of Summit Clinical Research for Summit networks sites management and support, Amrita Basu, Sam Anderson, and the rest of Agilex Biolabs, Ltd Pty. team for the sample bioanalyses, Christian Laveille, Stéphanie Blaizot and the rest of Calvagone Sarl. for the PK and PD analyses and population PK/PD modelling, Adriana Marinescu, Marion Odoul, Nathalie Roux-Drevet, Virginie Le Meaux, and the rest of ENYO Pharma team for the overall management and support.

Medical writing support provided by Dr. Lynsey Meikle PhD of Terminal 4 Communications and was supported financially by ENYO Pharma, Inc., Lyon, France.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.10.023.

References

Author names in bold designate shared co-first authorship.

[1] Loomba R, Friedman SL, Shulman GI. Mechanisms and disease consequences of nonalcoholic fatty liver disease. Cell 2021;184:2537–2564.
[2] Burra P, Becchetti C, Germani G. NAFLD and liver transplantation: disease burden, current management and future challenges. JHEP Rep 2020;2:100192.
[3] Noureddin M, Vipani A, Bresee C, Todo T, Kim IK, Alkhouri N, et al. NASH leading cause of liver transplant in women: updated analysis of indications for liver transplant and ethnic and gender variances. Am J Gastroenterol 2018;113:1649–1659.
[4] Harrison SA, Gawrieh S, Roberts K, Lisanti CJ, Schwope RB, Cebe KM, et al. Prospective evaluation of the prevalence of non-alcoholic fatty liver disease and steatohepatitis in a large middle-aged US cohort. J Hepatol 2021;75:284–291.
[5] Byrne CD, Targher G. NAFLD as a driver of chronic kidney disease. J Hepatol 2020;72:785–801.
[6] Sinn DH, Kang D, Jang HR, Gu S, Cho SJ, Paik SW, et al. Development of chronic kidney disease in patients with non-alcoholic fatty liver disease: a cohort study. J Hepatol 2017;67:1274–1280.
[7] Cao Y, Deng Y, Wang J, Zhao H, Zhang J, Xie W. The association between NAFLD and risk of chronic kidney disease: a cross-sectional study. Ther Adv Chronic Dis 2021;12:20406223211048649.
[8] Hara M, Tanaka S, Torisu K, Matsukuma Y, Tsuchimoto A, Tokumoto M, et al. Non-invasive fibrosis assessments of non-alcoholic fatty liver disease associated with low estimated glomerular filtration rate among CKD patients: the Fukuoka Kidney disease Registry Study. Clin Exp Nephrol 2021;25:822–834.
[9] Mantovani A, Petracca G, Beatrice G, Csermely A, Lonardo A, Schattenberg JM, et al. Non-alcoholic fatty liver disease and risk of incident chronic kidney disease: an updated meta-analysis. Gut 2022;71:156–162.
[10] Ratziu V, Francque S, Sanyal A. Breakthroughs in therapies for NASH and remaining challenges. J Hepatol 2022;76:1263–1278.
[11] Bishop-Bailey D, Walsh DT, Warner TD. Expression and activation of the farnesoid X receptor in the vasculature. Proc Natl Acad Sci U S A 2004;101:3668–3673.
[12] Hageman J, Herrema H, Groen AK, Kuipers F. A role of the bile salt receptor FXR in atherosclerosis. Arterioscler Thromb Vasc Biol 2010;30:1519–1528.
[13] Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet 2015;385:956–965.
[14] **Younossi ZM**, **Ratziu V**, Loomba R, Rinella M, Anstee QM, Goodman Z, et al. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. Lancet 2019;394:2184–2196.
[15] Siddiqui MS, Van Natta ML, Connelly MA, Vuppalanchi R, Neuschwander-Tetri BA, Tonascia J, et al. Impact of obeticholic acid on the lipoprotein profile in patients with non-alcoholic steatohepatitis. J Hepatol 2020;72:25–33.
[16] Al-Dury S, Wahlstrom A, Panzitt K, Thorell A, Stahlman M, Trauner M, et al. Obeticholic acid may increase the risk of gallstone formation in susceptible patients. J Hepatol 2019;71:986–991.
[17] Kleiner DE, Brunt EM, Wilson LA, Behling C, Guy C, Contos M, et al. Association of histologic disease activity with progression of nonalcoholic fatty liver disease. JAMA Netw Open 2019;2:e1912565.
[18] Caussy C, Reeder SB, Sirlin CB, Loomba R. Noninvasive, quantitative assessment of liver fat by MRI-PDFF as an endpoint in NASH trials. Hepatology 2018;68:763–772.
[19] Pavlides M, Banerjee R, Sellwood J, Kelly CJ, Robson MD, Booth JC, et al. Multiparametric magnetic resonance imaging predicts clinical outcomes in patients with chronic liver disease. J Hepatol 2016;64:308–315.
[20] Banerjee R, Pavlides M, Tunnicliffe EM, Piechnik SK, Sarania N, Philips R, et al. Multiparametric magnetic resonance for the non-invasive diagnosis of liver disease. J Hepatol 2014;60:69–77.
[21] Elman S, Hynan LS, Gabriel V, Mayo MJ. The 5-D itch scale: a new measure of pruritus. Br J Dermatol 2010;162:587–593.
[22] Reich A, Heisig M, Phan NQ, Taneda K, Takamori K, Takeuchi S, et al. Visual analogue scale: evaluation of the instrument for the assessment of pruritus. Acta Derm Venereol 2012;92:497–501.
[23] Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New creatinine- and cystatin C-based equations to estimate GFR without race. N Engl J Med 2021;385:1737–1749.
[24] Delgado C, Baweja M, Crews DC, Eneanya ND, Gadegbeku CA, Inker LA, et al. A unifying approach for GFR estimation: recommendations of the NKF-ASN task force on reassessing the inclusion of race in diagnosing kidney disease. Am J Kidney Dis 2022;79:268–288 e261.
[25] Jayaswal ANA, Levick C, Selvaraj EA, Dennis A, Booth JC, Collier J, et al. Prognostic value of multiparametric magnetic resonance imaging, transient elastography and blood-based fibrosis markers in patients with chronic liver disease. Liver Int 2020;40:3071–3082.
[26] Pedrosa M, Seyedkazemi S, Francque S, Sanyal A, Rinella M, Charlton M, et al. A randomized, double-blind, multicenter, phase 2b study to evaluate the safety and efficacy of a combination of tropifexor and cenicriviroc in patients with nonalcoholic steatohepatitis and liver fibrosis: study design of the TANDEM trial. Contemp Clin Trials 2020;88:105889.
[27] Patel K, Harrison SA, Elkashab M, Trotter JF, Herring R, Rojter S, et al. Cilofexor, a nonsteroidal FXR agonist, in non-cirrhotic patients with nonalcoholic steatohepatitis: a phase 2 randomized controlled trial. Hepatology 2020;72:58–71.
[28] Stine JG, Munaganuru N, Barnard A, Wang JL, Kaulback K, Argo CK, et al. Change in MRI-PDFF and histologic response in patients with nonalcoholic steatohepatitis: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2021;19:2274–2283.e2275.
[29] Tamaki N, Munaganuru N, Jung J, Yonan AQ, Loomba RR, Bettencourt R, et al. Clinical utility of 30% relative decline in MRI-PDFF in predicting fibrosis regression in non-alcoholic fatty liver disease. Gut 2022;71:983–990.
[30] Lucas KJ, Lopez P, Lawitz E, Sheikh A, Aizenberg D, Hsia S, et al. Safety and efficacy of tropifexor in patients with fibrotic nonalcoholic steatohepatitis: 48 week results from Part C of the Phase 2 FLIGHT FXR study. Hepatology 2020;72:LO139.
[31] Ratziu V, Rinella ME, Neuschwander-Tetri BA, Lawitz E, Denham D, Kayali Z, et al. EDP-305 in patients with NASH: a phase II double-blind placebo-controlled dose-ranging study. J Hepatol 2022;76:506–517.
[32] Wong VW. Predicting NASH response with liver fat: are we back to square one? J Hepatol 2020;72:386–388.
[33] Harrison SA, Neff G, Guy CD, Bashir MR, Paredes AH, Frias JP, et al. Efficacy and safety of aldafermin, an engineered FGF19 analog, in a randomized, double-blind, placebo-controlled trial of patients with nonalcoholic steatohepatitis. Gastroenterology 2021;160:219–231 e211.
[34] Harrison SA, Rinella ME, Abdelmalek MF, Trotter JF, Paredes AH, Arnold HL, et al. NGM282 for treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2018;391:1174–1185.
[35] Loomba R, Sanyal AJ, Kowdley KV, Terrault N, Chalasani NP, Abdelmalek MF, et al. Factors associated with histologic response in adult patients with nonalcoholic steatohepatitis. Gastroenterology 2019;156:88–95 e85.
[36] Bril F, Barb D, Lomonaco R, Lai J, Cusi K. Change in hepatic fat content measured by MRI does not predict treatment-induced histological improvement of steatohepatitis. J Hepatol 2020;72:401–410.
[37] Harrison SA, Bashir M, Moussa SE, McCarty K, Pablo Frias J, Taub R, et al. Effects of resmetirom on noninvasive endpoints in a 36-week phase 2 active treatment extension study in patients with NASH. Hepatol Commun 2021;5:573–588.
[38] Clifford BL, Sedgeman LR, Williams KJ, Morand P, Cheng A, Jarrett KE, et al. FXR activation protects against NAFLD via bile-acid-dependent reductions in lipid absorption. Cell Metab 2021;33:1671–1684 e1674.
[39] Pavlides M, Banerjee R, Tunnicliffe EM, Kelly C, Collier J, Wang LM, et al. Multiparametric magnetic resonance imaging for the assessment of non-alcoholic fatty liver disease severity. Liver Int 2017;37:1065–1073.
[40] Dennis A, Kelly MD, Fernandes C, Mouchti S, Fallowfield JA, Hirschfield G, et al. Correlations between MRI biomarkers PDFF and cT1 with histopathological features of non-alcoholic steatohepatitis. Front Endocrinol (Lausanne) 2020;11:575843.
[41] Andersson A, Kelly M, Imajo K, Nakajima A, Fallowfield JA, Hirschfield G, et al. Clinical utility of magnetic resonance imaging biomarkers for identifying nonalcoholic steatohepatitis patients at high risk of progression: a multicenter pooled data and meta-analysis. Clin Gastroenterol Hepatol 2021;20:2451–2461.
[42] Loomba R, Anstee QM, Harrison S, Sanyal A, Ratziu V, Younossi Z, et al. Obeticholic acid improves hepatic fibroinflammation as assessed by multiparametric magnetic resonance imaging: interim results of the REGENERATE trial. Gastroenterology 2020;158. S-1.
[43] Ali SS, Oni ET, Blaha MJ, Veledar E, Feiz HR, Feldman T, et al. Elevated gamma-glutamyl transferase is associated with subclinical inflammation independent of cardiometabolic risk factors in an asymptomatic population: a cross-sectional study. Nutr Metab (Lond) 2016;13:37.

[44] Ravuri C, Svineng G, Pankiv S, Huseby NE. Endogenous production of reactive oxygen species by the NADPH oxidase complexes is a determinant of gamma-glutamyltransferase expression. Free Radic Res 2011;45:600–610.
[45] Huang DQ, Sharpton SR, Amangurbanova M, Tamaki N, Sirlin CB, Loomba R, et al. Clinical utility of combined MRI-PDFF and ALT response in predicting histologic response in nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2022. In press.
[46] White CA, Akbari A, Allen C, Day AG, Norman PA, Holland D, et al. Simultaneous glomerular filtration rate determination using inulin, iohexol, and (99m)Tc-DTPA demonstrates the need for customized measurement protocols. Kidney Int 2021;99:957–966.
[47] Speeckaert MM, Seegmiller J, Glorieux G, Lameire N, Van Biesen W, Vanholder R, et al. Measured glomerular filtration rate: the query for a workable golden standard technique. J Pers Med 2021:11.
[48] Li S, Ghoshal S, Sojoodi M, Arora G, Masia R, Erstad DJ, et al. The farnesoid X receptor agonist EDP-305 reduces interstitial renal fibrosis in a mouse model of unilateral ureteral obstruction. FASEB J 2019;33:7103–7112.
[49] Zhao K, He J, Zhang Y, Xu Z, Xiong H, Gong R, et al. Activation of FXR protects against renal fibrosis via suppressing Smad3 expression. Sci Rep 2016;6:37234.
[50] Zhang Y, Xu Y, Qi Y, Xu L, Song S, Yin L, et al. Protective effects of dioscin against doxorubicin-induced nephrotoxicity via adjusting FXR-mediated oxidative stress and inflammation. Toxicology 2017;378:53–64.
[51] Bae EH, Choi HS, Joo SY, Kim IJ, Kim CS, Choi JS, et al. Farnesoid X receptor ligand prevents cisplatin-induced kidney injury by enhancing small heterodimer partner. PLoS One 2014;9:e86553.
[52] Gai Z, Chu L, Xu Z, Song X, Sun D, Kullak-Ublick GA. Farnesoid X receptor activation protects the kidney from ischemia-reperfusion damage. Sci Rep 2017;7:9815.
[53] Deprince A, Haas JT, Staels B. Dysregulated lipid metabolism links NAFLD to cardiovascular disease. Mol Metab 2020;42:101092.
[54] Chavez-Talavera O, Tailleux A, Lefebvre P, Staels B. Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease. Gastroenterology 2017;152:1679–1694 e1673.
[55] Pockros PJ, Fuchs M, Freilich B, Schiff E, Kohli A, Lawitz EJ, et al. CONTROL: a randomized phase 2 study of obeticholic acid and atorvastatin on lipoproteins in nonalcoholic steatohepatitis patients. Liver Int 2019;39:2082–2093.
[56] Harrison SA, Bashir MR, Lee KJ, Shim-Lopez J, Lee J, Wagner B, et al. A structurally optimized FXR agonist, MET409, reduced liver fat content over 12 weeks in patients with non-alcoholic steatohepatitis. J Hepatol 2021;75:25–33.
[57] Papazyan R, Liu X, Liu J, Dong B, Plummer EM, Lewis 2nd RD, et al. FXR activation by obeticholic acid or nonsteroidal agonists induces a human-like lipoprotein cholesterol change in mice with humanized chimeric liver. J Lipid Res 2018;59:982–993.
[58] Hambruch E, Miyazaki-Anzai S, Hahn U, Matysik S, Boettcher A, Perovic-Ottstadt S, et al. Synthetic farnesoid X receptor agonists induce high-density lipoprotein-mediated transhepatic cholesterol efflux in mice and monkeys and prevent atherosclerosis in cholesteryl ester transfer protein transgenic low-density lipoprotein receptor (-/-) mice. J Pharmacol Exp Ther 2012;343:556–567.
[59] Hartman HB, Gardell SJ, Petucci CJ, Wang S, Krueger JA, Evans MJ. Activation of farnesoid X receptor prevents atherosclerotic lesion formation in LDLR-/- and apoE-/- mice. J Lipid Res 2009;50:1090–1100.
[60] Mencarelli A, Renga B, Distrutti E, Fiorucci S. Antiatherosclerotic effect of farnesoid X receptor. Am J Physiol Heart Circ Physiol 2009;296:H272–H281.
[61] Younossi ZM, Stepanova M, Nader F, Loomba R, Anstee QM, Ratziu V, et al. Obeticholic acid impact on quality of life in patients with nonalcoholic steatohepatitis: REGENERATE 18-month interim analysis. Clin Gastroenterol Hepatol 2021;20:2050–2058.
[62] Younossi ZM, Stepanova M, Noureddin M, Kowdley KV, Strasser SI, Kohli A, et al. Improvements of fibrosis and disease activity are associated with improvement of patient-reported outcomes in patients with advanced fibrosis due to nonalcoholic steatohepatitis. Hepatol Commun 2021;5:1201–1211.