Volixibat in adults with non-alcoholic steatohepatitis

Volixibat in Adults study group

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Research Article
NAFLD and Alcohol-Related Liver Diseases

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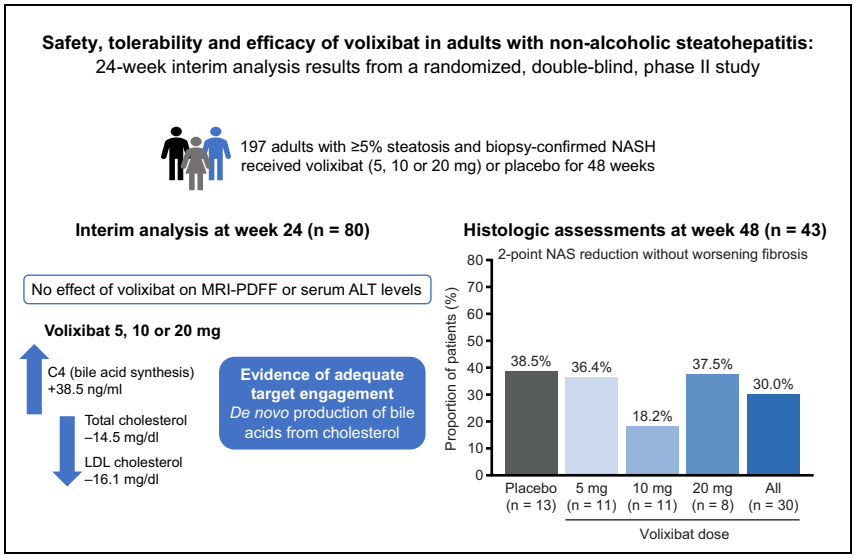
Graphical abstract



Highlights

- Volixibat decreased serum C4 (bile acid synthesis biomarker) and cholesterol, indicating adequate target engagement.
- Volixibat had no therapeutic impact on steatosis or liver injury in NASH.
- Treatment-emergent adverse events were mainly of mild or moderate grade.
- No serious adverse events were attributed to volixibat.

Authors

Philip N. Newsome, Melissa Palmer, Bradley Freilich, …, Tarek Hassanein, Hak-Myung Lee, Guruprasad P. Aithal

Correspondence

p.n.newsome@bham.ac.uk
(P.N. Newsome).

Lay summary

A medicine called volixibat has previously been shown to reduce cholesterol levels in the blood. This study investigated whether volixibat could reduce the amount of fat in the liver and reduce liver injury in adults with an advanced form of non-alcoholic fatty liver disease. Volixibat did not reduce the amount of fat in the liver, nor did it have any other beneficial effect on liver injury. Participants in the study generally tolerated the side effects of volixibat and, as in previous studies, the main side effect was diarrhoea. These results show that volixibat is not an effective treatment for people with fatty liver disease.

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Volixibat in adults with non-alcoholic steatohepatitis: 24-week interim analysis from a randomized, phase II study

Philip N. Newsome[1,2,*], Melissa Palmer[3], Bradley Freilich[4], Muhammad Y. Sheikh[5], Aasim Sheikh[6], Harry Sarles[7], Robert Herring[8], Parvez Mantry[9], Zeid Kayali[10], Tarek Hassanein[11], Hak-Myung Lee[3], Guruprasad P. Aithal[12,13], on behalf of the Volixibat in Adults study group

[1]National Institute for Health Research, Birmingham Biomedical Research Centre at the University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK; [2]Centre for Liver and Gastrointestinal Research, Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, UK; [3]Shire Plc, a Takeda company, Lexington, MA, USA; [4]Kansas City Research Institute, Kansas City, MO, USA; [5]Fresno Clinical Research Center, Fresno, CA, USA; [6]GI Specialists of Georgia, Marietta, GA, USA; [7]DHAT Research Institute, Garland, TX, USA; [8]Quality Medical Research, PLLC, Nashville, TN, USA; [9]Methodist Health System Clinical Research Institute, Dallas, TX, USA; [10]Inland Empire Liver Foundation, Rialto, CA, USA; [11]Southern California Research Center, Coronado, CA, USA; [12]National Institute for Health Research, Nottingham Biomedical Research Centre at the Nottingham University Hospitals NHS Trust and the University of Nottingham, Nottingham, UK; [13]Nottingham Digestive Diseases Centre, School of Medicine, University of Nottingham, Nottingham, UK

Background & Aims: Volixibat is an inhibitor of the apical sodium-dependent bile acid transporter (ASBT) that has been hypothesized to improve non-alcoholic steatohepatitis (NASH) by blocking bile acid reuptake and stimulating hepatic bile acid production. We studied the safety, tolerability and efficacy of volixibat in patients with NASH.

Methods: In this double-blind, phase II dose-finding study, adults with ≥5% steatosis and NASH without cirrhosis (N = 197) were randomized to receive volixibat (5, 10 or 20 mg) or placebo once daily for 48 weeks. The endpoints of a pre-defined interim analysis (n = 80), at week 24, were: ≥5% reduction in MRI-proton density fat fraction and ≥20% reduction in serum alanine aminotransferase levels. The primary endpoint was a ≥2-point reduction in non-alcoholic fatty liver disease activity score without worsening fibrosis at week 48.

Results: Volixibat did not meet either interim endpoint; the study was terminated owing to lack of efficacy. In participants receiving any volixibat dose, mean serum 7-alpha-hydroxy-4-cholesten-3-one (C4; a biomarker of bile acid synthesis) increased from baseline to week 24 (+38.5 ng/ml [SD 53.18]), with concomitant decreases in serum total cholesterol (−14.5 mg/dl [SD 28.32]) and low-density lipoprotein cholesterol (−16.1 mg/dl [SD 25.31]). These changes were generally dose-dependent. On histological analysis, a greater proportion of participants receiving placebo (38.5%, n = 5/13) than volixibat (30.0%, n = 9/30) met the primary endpoint. Treatment-emergent adverse events (TEAEs) were mainly mild or moderate. No serious TEAEs were related to volixibat. Diarrhoea was the most common TEAE overall and the most common TEAE leading to discontinuation.

Conclusions: Increased serum C4 and decreased serum cholesterol levels provide evidence of target engagement. However, inhibition of ASBT by volixibat did not elicit a liver-related therapeutic benefit in adults with NASH.

Lay summary: A medicine called volixibat has previously been shown to reduce cholesterol levels in the blood. This study investigated whether volixibat could reduce the amount of fat in the liver and reduce liver injury in adults with an advanced form of non-alcoholic fatty liver disease. Volixibat did not reduce the amount of fat in the liver, nor did it have any other beneficial effect on liver injury. Participants in the study generally tolerated the side effects of volixibat and, as in previous studies, the main side effect was diarrhoea. These results show that volixibat is not an effective treatment for people with fatty liver disease.

Clinical trial identifier: NCT02787304.

Introduction

Non-alcoholic steatohepatitis (NASH) is a progressive form of non-alcoholic fatty liver disease (NAFLD), characterized by the hepatic accumulation of fat (steatosis), inflammation and hepatocellular injury (ballooning), with or without progressive fibrosis. NASH can lead to cirrhosis, liver failure and liver cancer.[1–3] Large meta-analyses demonstrate that NASH progresses faster and is associated with greater liver-related and overall mortality than NAFLD.[4,5] The prevalence of NASH is difficult to determine because it is often asymptomatic and because a definitive diagnosis requires a liver biopsy for histologic examination.[1,6,7] An estimated 12.2% of middle-aged adults may have NASH in the USA, rising to 22% among those with diabetes and to 33% among those with obesity.[7,8] In 2014, NASH surpassed chronic hepatitis C as the leading indication

Keywords: Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Steatosis; Alanine aminotransferase; ASBT inhibitor; Humans; Phase II.
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* Corresponding author. Address: NIHR Birmingham Biomedical Research Centre, University of Birmingham, Birmingham, UK. Tel.: 0121 415 8700.
E-mail address: p.n.newsome@bham.ac.uk (P.N. Newsome).

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for liver transplantation in adults younger than 50 years of age in the USA[9]; in Europe, the proportion of liver transplants attributed to NASH increased from 1.2% in 2002 to 8.4% in 2016.[10]

There is currently no marketed pharmacological treatment for NASH.[11] Clinical practice guidelines for the management of NAFLD recommend behavioural change intended to reduce body weight through dietary restriction and physical activity.[12] However, such interventions are often limited in their therapeutic effect owing to poor long-term adherence and considerable rates of weight regain.[13,14] Therefore, there is a significant unmet need for an effective pharmacological treatment for patients with NASH.

Abnormal cholesterol metabolism and accumulation of free cholesterol in the liver contribute directly to the pathogenesis of NASH[15,16] because free cholesterol is toxic to hepatocytes, driving development of inflammation and fibrosis.[17] Volixibat potassium (SHP626; formerly LUM002; hereafter referred to as volixibat) is a highly selective, minimally absorbed competitive inhibitor of the apical sodium-dependent bile acid transporter (ASBT). ASBT inhibition may represent a strategy for therapeutic intervention in patients with NASH, owing to its effects on cholesterol metabolism.[18] ASBT is localized primarily on the luminal surface of ileal enterocytes and selectively reabsorbs bile acids (BAs) during enterohepatic recirculation.[19,20] Inhibition of ASBT prevents reabsorption of BAs from the intestinal lumen, thereby increasing faecal BA excretion and reducing recirculation of BAs to the liver via the hepatic portal vein. Reduced recirculation of BAs stimulates *de novo* hepatic BA production from free cholesterol that is present in the liver and the bloodstream.[21,22] Consistent with this mechanism of action, increases in serum levels of 7α-hydroxy-4-cholesten-3-one (7αC4, also known as C4; a biomarker of BA synthesis) and decreases in serum cholesterol levels are observed following administration of ASBT inhibitors, including volixibat.[23–27]

In the treatment of NASH, volixibat may reduce the pathogenic accumulation of cholesterol in the liver by reducing the levels of BAs returning to the liver via enterohepatic recirculation and by stimulating *de novo* production of BAs from free cholesterol in the liver and serum. This may have therapeutically beneficial anti-inflammatory, anti-steatotic and anti-fibrotic effects. Volixibat may also have positive metabolic effects because BAs act as signalling molecules that play a role in glucose metabolism pathways, including hepatic gluconeogenesis, glycogen synthesis and insulin sensitivity.[28,29] In mice that were fed a high-fat diet, administration of SC-435 (a surrogate of volixibat) significantly reduced hepatic concentrations of triglycerides, cholesteryl ester and total cholesterol to levels that were comparable to those of standard chow-fed mice.[30] In addition, ASBT inhibition with SC-435 or volixibat restored glucose tolerance and significantly decreased NAFLD activity score (NAS) and hepatocellular hypertrophy.[30,31] SC-435 also blocked progression of sclerosing cholangitis and reduced hepatic fibrosis in *Mdr2*$^{-/-}$ knockout mice.[32] Phase I studies have demonstrated that volixibat is not metabolized, is minimally absorbed and is effective at reducing serum cholesterol in overweight and obese adults.[18,26,27] The increases in faecal BA excretion and serum C4 levels observed in these phase I studies support the proposed mechanism of action of volixibat in patients with NASH. It is also important that volixibat was found to reduce fasting glucose levels significantly compared with placebo in patients with type 2 diabetes mellitus (T2DM)[26] because there is a close association between NASH and T2DM.[33–35]

Herein, we report the 24-week interim analysis of a 48-week, phase II, proof-of-concept study of volixibat in adults with NASH. We also report the histologic analyses of participants who completed the study to week 48.

Patients and methods

Overview

This was a randomized, double-blind, phase II, placebo-controlled, parallel-group, proof-of-concept, dose-finding study of volixibat in adults with NASH (ClinicalTrials.gov identifier: NCT02787304). All participants provided written informed consent. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the independent ethics committee of each study site.

Participants

Eligible participants were 18–80 years of age, with at least 5% steatosis on centrally read MRI-proton density fat fraction (MRI-PDFF) scans and with centrally read histologic confirmation of NASH without cirrhosis (fibrosis stage F0–F3).[36] Participants were required to have a NAS of at least 4 points, with a score of at least 1 point in each NAS component (steatosis, lobular inflammation and hepatocyte ballooning).

Patients were excluded if they had a history or presence of concomitant liver disease (*e.g.* decompensated liver disease, hepatocellular carcinoma) or any other current or recurrent disease that may affect the action or assessment of volixibat (including, but not limited to, uncontrolled inflammatory bowel disease, uncontrolled coeliac disease, gastric bypass or history of chronic diarrhoea). Participants were also ineligible if they had: type 1 diabetes mellitus or uncontrolled T2DM (defined as glycated haemoglobin levels of ≥9.5% in the 60 days before enrolment); serum alanine aminotransferase (ALT) or aspartate aminotransferase levels at least 7× the upper limit of normal (ULN) at screening (normal range of ALT: 0–55 U/L); or a known history of alcohol or other substance abuse in the past year or at any time during the study.

Design

The study was conducted at 68 centres (53 in the USA, 6 in Canada and 9 in the UK) from October 2016 to July 2018. The predefined interim safety, tolerability and efficacy analysis was conducted in June 2018. Following the interim analysis, the study was terminated early owing to lack of efficacy.

The study comprised a 10-week screening period, a 48-week treatment period and a 4-week follow-up. Participants were scheduled to attend the clinic at screening, at baseline, at weeks 2, 4, 8, 12, 24, 36 and 48 of treatment, and at week 52 for follow-up. After screening, participants were randomized 1:1:1:1 to either 5, 10 or 20 mg of volixibat or placebo, administered orally once daily in a double-blind fashion using blinded blister packs. Doses were chosen based on phase I data.[27] Participants were automatically randomized to treatment groups based on an interactive response technology, stratified by the presence of T2DM and baseline NAS (divided into NAS = 4–5 or NAS = 6–8).

Outcome measures

The primary objective of the study was to examine the effect of volixibat compared with placebo on liver histology at week 48. Secondary objectives included the effect of volixibat on hepatic steatosis, assessed by MRI-PDFF, and serum ALT levels.

Week 24 interim analysis

The interim analysis of the safety, tolerability and efficacy of volixibat was prespecified to occur when 80 participants had received 24 weeks of treatment. In this interim analysis, clinically significant efficacy of volixibat was assessed based on predefined endpoints of absolute reduction from baseline to week 24 in steatosis of at least 5%, as assessed by MRI-PDFF, and relative reduction from baseline to week 24 in serum ALT levels of at least 20%. BA synthesis was assessed via serum C4 concentration, as an exploratory pharmacodynamic outcome. Changes in serum glucose levels, serum lipid levels, body weight and waist-to-hip ratio were examined as additional interim endpoints.

Week 48 analyses

Week 48 analyses were conducted using data from participants who had reached this time point when the study was terminated. The prespecified primary endpoint was a reduction of at least 2 points in NAS, without worsening fibrosis, from baseline to week 48. A *post hoc* decision was made following early termination of the study to analyse the prespecified histologic outcomes in participants who had paired liver biopsies at screening and at week 48. Secondary histologic endpoints were: decrease in fibrosis stage, irrespective of NAS; resolution of NASH (defined as absence of ballooning [score = 0] and absent or mild inflammation [score 0–1], with or without steatosis) without worsening fibrosis; and change in liver histology, as measured by the individual NAS components (ballooning, inflammation and steatosis).

Changes in hepatic steatosis, as measured by MRI-PDFF, and serum ALT levels were assessed as additional secondary endpoints at week 48.

Assessments

Steatosis was assessed at screening and during weeks 24 and 48 clinic visits with a centrally read MRI-PDFF, based on images from multi-echo and double-echo sequences. Two liver biopsies were required for histologic analyses. The first was scheduled during the screening visit, unless participants had a liver biopsy available up to 6 months before screening. The second biopsy was taken upon study completion at week 48. Liver biopsies were centrally read by a NASH Clinical Research Network pathologist for confirmation of the diagnosis of NASH and for assessment and grading of NAS (steatosis, lobular inflammation and ballooning) and stage of fibrosis (F0–F3). Serum ALT, serum C4 and metabolic indicators (serum glucose and serum cholesterol) were also assessed at scheduled clinic visits.

Treatment-emergent adverse events (TEAEs) were monitored at all study visits. Stool hardness was assessed by recording the softest evacuation during the 24 h before each clinic visit using the Bristol Stool Chart. Stool frequency was assessed by the number of bowel movements during the 24 h before each clinic visit, and a frequency of 6 or more bowel movements per day was deemed potentially clinically important. Vital signs, weight, waist-to-hip ratio, electrocardiogram and clinical laboratory tests (chemistry, haematology, coagulation and urinalysis) were also monitored at scheduled visits.

Sample size calculation

Sample size calculations were based on expected response rates of 21% in the placebo group and 45% in the active group in the primary efficacy outcome.[37] To achieve 80% power with a 10% type I error, 67 participants per treatment group were required to complete the study. Owing to early termination, the study did not meet the target sample size. Therefore, all results are descriptive and non-inferential.

Interim statistical analyses

The safety set included all participants who were randomized, had taken at least 1 dose of volixibat or placebo and had at least 1 post-baseline safety assessment. The full analysis set included all participants in the safety set who had at least 1 post-baseline efficacy assessment. The interim analysis set included all participants in the safety set who had both baseline and scheduled week 24 efficacy assessment (MRI-PDFF and ALT) at the time of the interim analysis. The pharmacodynamic set included all participants who had provided at least 1 blood sample after receiving their first dose of volixibat or placebo.

Dose-selection analyses were conducted at the interim analysis to determine which volixibat dose or doses were to be discontinued based on efficacy, safety and tolerability. The probability of a clinically important effect on MRI-PDFF (≥5% absolute reduction) and on serum ALT (≥20% reduction) was calculated from the posterior distribution of Bayesian hierarchical models, which modelled changes in these outcomes across the 3 volixibat arms. Doses were not investigated further if the probability of a clinically important effect for both MRI-PDFF and ALT levels was less than or equal to 10%. Volixibat doses were to be discontinued based on poor tolerability if 6 or more additional participants discontinued the study owing to any particular TEAE compared with the placebo group. If volixibat doses were deemed unsafe based on clinical judgement, then randomization to that dose was discontinued.

Interim endpoints were stratified into subgroups of sex, presence of T2DM, baseline NAS and stage of fibrosis *post hoc*.

Results

Participant disposition

In total, 585 individuals were screened, and 197 participants were randomized to receive volixibat 5 mg (n = 49), volixibat 10 mg (n = 50), volixibat 20 mg (n = 49) or placebo (n = 49; Fig. 1). One participant in the volixibat 10 mg group was lost to follow-up before receiving treatment; therefore, 196 participants were included in the safety set. Eighty participants received at least 24 weeks of treatment with volixibat 5 mg (n = 21), volixibat 10 mg (n = 20), volixibat 20 mg (n = 18) or placebo (n = 21) and were included in the interim analysis. At the time of the interim analysis, 48 participants had completed the study and provided data to week 48, and 43 had paired liver biopsies at screening and at week 48 (volixibat 5 mg, n = 11; volixibat 10 mg, n = 11; volixibat 20 mg, n = 8; placebo, n = 13).

Following the interim analysis, the study was terminated owing to lack of efficacy. Of the 196 participants who received a

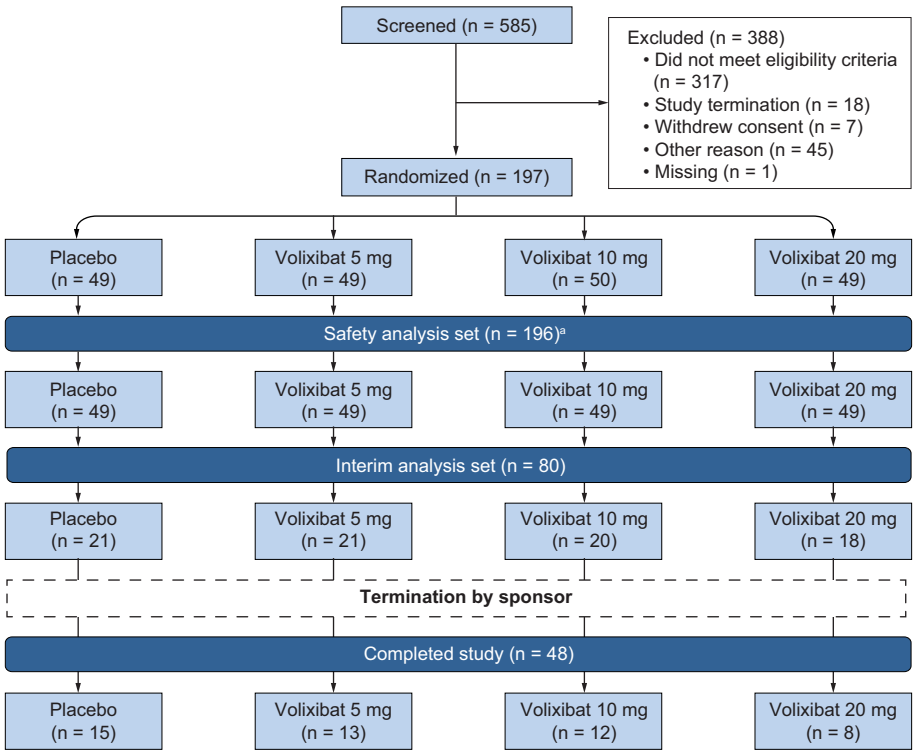

Fig. 1. Participant disposition. [a]The safety set included 196 participants because 1 of the 197 participants who were randomized to the volixibat 10 mg group was lost to follow-up before receiving treatment.

dose of volixibat or placebo, most (75.5%; n = 148) did not complete the study, mainly owing to termination of the study by the sponsor (60.7%) or to TEAEs (10.7%). The most common TEAE leading to study withdrawal was diarrhoea (7.1%).

Demographic and baseline characteristics

Demographic and baseline characteristics were generally well balanced between the treatment groups (Table 1). The 196 participants in the safety set had a mean age of 53.1 years (SD 12.78). Most participants were white (89.3%), were female (60.2%) and had a body mass index (BMI) in the obese range (73.5%), with a mean BMI of 34.5 kg/m^2 (SD 6.35). Overall, 43.4% of participants had T2DM.

At baseline, mean NAS was 5.2 points (SD 1.08), mean MRI-PDFF was 18.5% (SD 8.24), mean serum C4 level was 39.7 ng/ml (SD 35.56) and median serum ALT level was 49.5 (IQR 42.5). Mean baseline NAS, MRI-PDFF and C4 levels and median ALT levels were similar across all treatment groups, as was the proportion of participants with ALT levels in the normal range (Table 1). Overall, 16.8% of participants had no fibrosis, 38.3% had stage 1a–c fibrosis, 13.3% had stage 2 fibrosis and 31.1% had stage 3 fibrosis (0.5% of participants did not have baseline fibrosis data).

Indicators of target engagement

Mean serum C4 concentrations increased from baseline to week 24 by 38.5 ng/ml (SD 53.18) in participants receiving any dose of volixibat, with no change in the placebo group (−3.2 ng/ml [SD 26.05]). Mean increases in serum C4 concentrations did not appear to be dose-dependent, whereas mean decreases in serum total cholesterol, low-density lipoprotein (LDL) cholesterol and glucose concentrations were dose-dependent (Table 2). Mean serum total cholesterol levels decreased from baseline to week 24 by 14.5 mg/dl (SD 28.32) in participants receiving any dose of volixibat and increased by 1.0 mg/dl (SD 24.64) in the placebo group. Mean serum LDL cholesterol levels decreased from baseline to week 24 by 16.1 mg/dl (SD 25.31) in participants receiving any dose of volixibat and decreased by 1.4 mg/dl (SD 18.97) in the placebo group. Mean serum cholesterol (total and LDL) and C4 levels returned to baseline by week 52 follow-up after treatment had stopped (Fig. S1). Mean change in serum glucose levels from baseline to week 24 was −3.0 mg/dl (SD 29.66) in participants receiving any dose of volixibat and +3.0 mg/dl (SD 34.43) in the placebo group.

Efficacy

At week 24, no dose of volixibat had a probability of a clinically important effect above 10% in the Bayesian model (Table 3), leading to early termination of the study.

MRI-PDFF

Change from baseline in MRI-PDFF did not meet the predefined criteria for clinically significant efficacy (≥5% absolute decrease) in any of the volixibat dose groups at week 24 (Table 3) or week 48 (Table S1). Mean absolute percentage changes from baseline to week 24 in MRI-PDFF were similar at all doses of volixibat: 5 mg (–0.4% [SD 5.73]), 10 mg (–0.2% [SD 7.91]) or 20 mg (–1.3% [SD 4.85]) *vs.* placebo (+0.2% [SD 5.11]). No improvements in MRI-

Table 1. Baseline demographic and disease characteristics.

		Volixibat				
	Placebo	5 mg	10 mg	20 mg	All doses	Total
Safety set	**n = 49**	**n = 49**	**n = 49**	**n = 49**	**n = 147**	**n = 196**
Age, years, mean (SD)	53.4 (11.75)	52.8 (14.13)	53.0 (11.84)	53.2 (13.61)	53.0 (13.14)	53.1 (12.78)
Sex, n (%)						
Male	17 (34.7)	22 (44.9)	15 (30.6)	24 (49.0)	61 (41.5)	78 (39.8)
Female	32 (65.3)	27 (55.1)	34 (69.4)	25 (51.0)	86 (58.5)	118 (60.2)
Ethnicity, n (%)						
Hispanic or Latino	7 (14.3)	9 (18.4)	10 (20.4)	7 (14.3)	26 (17.7)	33 (16.8)
Not Hispanic or Latino	42 (85.7)	40 (81.6)	39 (79.6)	42 (85.7)	121 (82.3)	163 (83.2)
Race, n (%)						
White	41 (83.7)	46 (93.9)	47 (95.9)	41 (83.7)	134 (91.2)	175 (89.3)
Non-white	8 (16.3)	3 (6.1)	2 (4.1)	8 (16.3)	13 (8.8)	21 (10.7)
Black or African American	4 (8.2)	0	1 (2.0)	4 (8.2)	5 (3.4)	9 (4.6)
Asian	4 (8.2)	2 (4.1)	1 (2.0)	4 (8.2)	7 (4.8)	11 (5.6)
Multiple	0	1 (2.0)	0	0	1 (0.7)	1 (0.5)
T2DM, n (%)						
Yes	21 (42.9)	22 (44.9)	21 (42.9)	21 (42.9)	64 (43.5)	85 (43.4)
No	28 (57.1)	27 (55.1)	28 (57.1)	28 (57.1)	83 (56.5)	111 (56.6)
NAS, mean (SD)	5.2 (0.96)	5.2 (1.01)	5.2 (1.26)	5.1 (1.11)	5.1(1.12)	5.2 (1.08)
Stage of fibrosis, n (%)						
0: none	11 (22.4)	7 (14.3)	7 (14.3)	8 (16.3)	22 (15.0)	33 (16.8)
1a: mild zone 3 perisinusoidal (requires trichome)	12 (24.5)	13 (26.5)	8 (16.3)	9 (18.4)	30 (20.4)	42 (21.4)
1b: moderate zone 3 perisinusoidal (visible on haematoxylin and eosin staining)	7 (14.3)	7 (14.3)	7 (14.3)	6 (12.2)	20 (13.6)	27 (13.8)
1c: portal/periportal only	0	3 (6.1)	2 (4.1)	1 (2.0)	6 (4.1)	6 (3.1)
2: portal, periportal and perisinusoidal	7 (14.3)	6 (12.2)	6 (12.2)	7 (14.3)	19 (12.9)	26 (13.3)
3: bridging	12 (24.5)	13 (26.5)	18 (36.7)	18 (36.7)	49 (33.3)	61 (31.1)
4: cirrhosis	0	0	0	0	0	0
Missing	0	0	1 (2.0)	0	1 (0.7)	1 (0.5)
Stage of fibrosis, mean (SD)[a]	1.4 (1.10)	1.5 (1.04)	1.7 (1.12)	1.7 (1.14)	1.6 (1.10)	1.6 (1.10)
Stage of fibrosis, median[a]	1	1	1	2	1	1
MRI-PDFF, %, mean (SD)	18.8 (8.78)	20.4 (7.60)	17.8 (8.72)	17.0 (7.66)	18.4 (8.08)	18.5 (8.24)
Interim analysis set	**n = 21**	**n = 21**	**n = 20**	**n = 18**	**n = 59**	**n = 80**
Serum ALT, U/L, median (IQR)	43.0 (36.0)	60.0 (48.0)	58.5 (50.5)	50.0 (32.0)	53.0 (47.0)	49.5 (42.5)
ALT levels within normal range, n (%)[b]	13 (61.9)	10 (47.6)	10 (50.0)	11 (61.1)	31 (52.5)	44 (55.0)
Pharmacodynamic set	**n = 49**	**n = 49**	**n = 47**	**n = 49**	**n = 145**	**n = 194**
Serum C4, ng/ml, mean (SD)	42.9 (33.19)	39.9 (29.73)	39.2 (36.36)	37.0 (42.86)	38.7 (36.37)	39.7 (35.56)

Percentages may not sum to 100 owing to rounding error.
ALT, alanine aminotransferase; C4, 7-alpha-hydroxy-4-cholesten-3-one; MRI-PDFF, MRI-proton density fat fraction; NAS, non-alcoholic fatty liver disease activity score; T2DM, type 2 diabetes mellitus.
[a]When computing mean/median stage of fibrosis, stages 1a, 1b and 1c were all taken as 1.
[b]Normal range of ALT: 0–55 U/L.

PDFF at week 24 were observed at any dose of volixibat in *post hoc* subgroup analyses (sex, presence of T2DM, baseline NAS or stage of fibrosis).

Serum ALT levels

Change from baseline in serum ALT levels did not meet the predefined criteria for clinically significant efficacy (≥20% decrease) in any of the volixibat dose groups at week 24 (Table 3) or week 48 (Table S1). Mean absolute change from baseline to week 24 in serum ALT levels was not different for any dose of volixibat: 5 mg (+6.9 U/L [SD 29.87]), 10 mg (+7.3 U/L [SD 42.90]) or 20 mg (−3.3 U/L [SD 26.20]) *vs.* placebo (−6.3 U/L [SD 30.36]). Overall, 3.8% of participants (1 participant per volixibat dose) had increases in ALT level greater than 3× the ULN. No

Table 2. Mean (SD) change from baseline to week 24 in serum C4 levels (pharmacodynamic set; N = 194) and in metabolic indicators (safety set; N = 196).

			Volixibat			
		Placebo	5 mg	10 mg	20 mg	All doses
Serum C4, ng/ml	n	34	34	31	29	94
		−3.2 (26.05)	32.8 (40.26)	53.7 (48.30)	28.8 (67.75)	38.5 (53.18)
Serum glucose, mg/dl	n	37	37	33	33	103
		3.0 (34.43)	−0.5 (28.64)	−3.7 (33.86)	−5.1 (26.87)	−3.0 (29.66)
Serum cholesterol, mg/dl	n	37	37	33	33	103
		1.0 (24.64)	−9.0 (20.17)	−12.7 (30.85)	−22.5 (32.29)	−14.5 (28.32)
Serum LDL cholesterol, mg/dl	n	37	37	33	33	103
		−1.4 (18.97)	−11.4 (21.09)	−15.0 (26.90)	−22.5 (27.36)	−16.1 (25.31)

C4, 7-alpha-hydroxy-4-cholesten-3-one; LDL, low-density lipoprotein.

Table 3. Dose-selection analyses and changes from baseline to week 24 in MRI-PDFF and serum ALT levels (interim analysis set; N = 80).

		Volixibat		
	Placebo (n = 21)	5 mg (n = 21)	10 mg (n = 20)	20 mg (n = 18)
Dose selection (Bayesian model)				
Absolute percentage change in MRI-PDFF				
Mean (95% CI)		−0.6 (−2.3, 1.2)	−0.6 (−2.3, 1.3)	−0.7 (−2.6, 1.1)
Futility threshold		−3.8	−3.8	−3.8
PCIE, %		0.0	0.0	0.0
Percentage change in ALT				
Mean (95% CI)		11.8 (−1.2, 24.5)	11.4 (−1.5, 23.8)	10.1 (−3.8, 22.4)
Futility threshold		−11.3	−11.4	−11.0
PCIE, %		0.0	0.0	0.1
Interim efficacy analysis				
Absolute percentage change in MRI-PDFF				
Mean (SD)	0.2 (5.11)	−0.4 (5.73)	−0.2 (7.91)	−1.3 (4.85)
Relative percentage change in ALT				
Mean (SD)	−0.0 (25.60)	17.1 (48.72)	14.1 (45.13)	2.4 (39.02)
Absolute change in ALT, U/L				
Mean (SD)	−6.3 (30.36)	6.9 (29.87)	7.3 (42.90)	−3.3 (26.20)

ALT, alanine aminotransferase; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; PCIE, probability of a clinically important effect.

participants had an increase in ALT levels of greater than 3× baseline. No improvement in serum ALT levels at week 24 was observed at any dose of volixibat in *post hoc* subgroup analyses (sex, presence of T2DM, baseline NAS or stage of fibrosis).

Liver histology at week 48

When the 3 histologic endpoints (≥2-point reduction in NAS without worsening fibrosis [primary efficacy endpoint], fibrosis reduction and NASH resolution without worsening fibrosis) were assessed in participants who had liver biopsies at baseline and week 48 (n = 43), response rates were generally higher in the placebo group than in the volixibat groups (Fig. 2). There were no notable changes in individual components of NAS at week 48 (Table S1). The stage of fibrosis at week 48 is also shown in Table S1.

In participants with a baseline fibrosis stage of 2 or higher (n = 23), response rates for each of the 3 histologic endpoints were generally higher in the placebo group (50.0–66.7%) than in the volixibat dose groups (16.7–66.7%) at week 48 (Fig. 2). In participants with a baseline NAS of 4 or more (n = 40), response rates for each of the 3 histologic endpoints were generally higher in the placebo group (33.3–41.7%) than in the volixibat dose groups (12.5–45.5%) at week 48.

Safety and tolerability

TEAEs were reported in 130/147 participants receiving volixibat (88.4%) and 37/49 participants receiving placebo (75.5%) (Table 4). Serious TEAEs were reported in 3/147 participants receiving volixibat (2.0%) and 1/49 participants receiving placebo (2.0%); all serious TEAEs were unrelated to volixibat, and no TEAEs resulted in death. Most TEAEs were mild or moderate in severity. Severe TEAEs were reported in 9/147 participants receiving volixibat (6.1%) and 2/49 participants receiving placebo (4.1%); the incidence of severe TEAEs was similar across the volixibat doses. TEAEs leading to discontinuation of treatment were reported in 20/147 participants receiving volixibat (13.6%) and 1/49 participants receiving placebo (2.0%).

Diarrhoea was the most common TEAE, occurring in a non-dose-dependent manner in 108/147 participants receiving volixibat (73.5%) and in 10/49 participants receiving placebo (20.4%). Diarrhoea was also the most common TEAE leading to discontinuation of treatment, occurring in a non-dose-dependent manner in 14/147 participants receiving volixibat (9.5%) and in no participants receiving placebo. Most diarrhoea occurred intermittently. Across the safety set, 86/196 participants (43.9%) had intermittent TEAEs of diarrhoea, and 41/196 participants (20.9%) had continuous TEAEs of diarrhoea (Fig. S2). Most diarrhoea TEAEs that led to discontinuation of volixibat (12/14 events) occurred in the first 2 weeks of treatment. The incidence of diarrhoea TEAEs decreased after week 2 (Fig. S3). Other common gastrointestinal TEAEs in participants receiving volixibat included abdominal pain (25/147; 17.0%), nausea (16/147; 10.9%) and vomiting (8/147; 5.4%), which occurred at slightly lower or similar rates in the placebo group.

Based on the Bristol Stool Chart, stools were softer during treatment with volixibat than with placebo. Most stools were classified as normal softness at the week 52 follow-up, once treatment had stopped (Table S2). Bowel movements were more frequent with volixibat than with placebo at week 2 but not at subsequent time points (Table S3). Changes in stool softness and bowel movement frequency were not dose-dependent.

There were no clear trends over time, or differences between treatment groups, in vital signs, physical findings (including waist-to-hip ratio), or electrocardiogram or laboratory data. There was a slight decrease in body weight in both the placebo and volixibat groups (mean change from baseline to week 24: placebo = −0.2 kg [SD 3.49]; volixibat = −1.4 kg [SD 3.90]).

Discussion

The ASBT inhibitor volixibat increased mean serum C4 and decreased serum total cholesterol levels, indicating target engagement, but it had no effect on steatosis, serum ALT levels or liver histology in adults with NASH in this randomized, double-blind, phase II, placebo-controlled, dose-finding study.

Based on previous clinical and preclinical studies, volixibat was hypothesized to exert therapeutic effects in patients

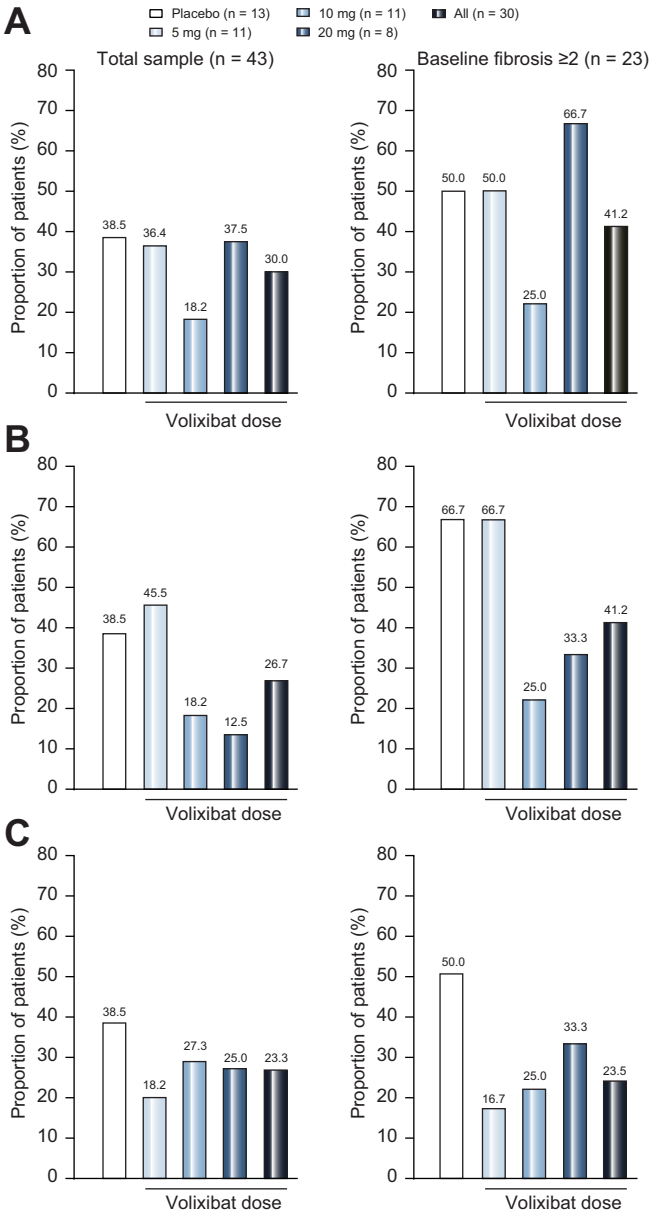

Fig. 2. Proportion of participants in the overall sample and with baseline fibrosis score ≥2 who met histological endpoints at week 48. (A) At least a 2-point reduction in NAS without worsening fibrosis; (B) fibrosis reduction; and (C) NASH resolution without worsening fibrosis. NAS, non-alcoholic fatty liver disease activity score; NASH, non-alcoholic steatohepatitis.

with NASH by blocking enterohepatic recirculation of BAs via inhibition of ASBT, thereby stimulating *de novo* production of BAs from cholesterol that is present in the liver and the bloodstream. The resulting reductions in systemic and hepatic cholesterol levels may then have anti-inflammatory, anti-steatotic and anti-fibrotic effects.[21–32] In this study, volixibat increased serum C4 concentrations and decreased serum cholesterol, indicating that BA synthesis from cholesterol was upregulated as a consequence of adequate target engagement.

Upregulation of BA synthesis and decreased serum cholesterol levels were not, however, accompanied by any clinically significant reduction in steatosis or serum ALT levels after 24 weeks of treatment. There was also no improvement in any liver histology outcome after 48 weeks of treatment, based on the subset of participants who had completed the study. The elevated serum C4 and decreased total and LDL cholesterol levels observed in this study are consistent with results from a phase I study in healthy volunteers.[27] Although these results suggest that inhibition of ASBT with volixibat monotherapy may increase BA synthesis from cholesterol, there does not appear to be any concomitant effect in reducing hepatic steatosis or injury, despite evidence for a mechanistic link in mouse models.[30,31] In mice that were fed a high-fat diet, statistically significant reductions in hepatocellular hypertrophy and NAS were accompanied by attenuated serum cholesterol levels, changes in BA metabolism and a trend towards a small decrease in steatosis following administration of volixibat.[31] Animal models may not accurately reflect the pathophysiology of NASH in humans. There is a possibility that the dose of volixibat or the duration of treatment was insufficient to mediate effects on steatosis in this study. However, the lack of consistent dose-dependent relationships in indicators of target engagement or efficacy outcomes suggests that an increased dose of volixibat might not have led to any greater target engagement. Similarly, after immediate improvement, serum cholesterol and C4 levels remained consistent throughout the study duration, indicating that participants would not have benefited from additional duration of treatment.

The safety profile of volixibat in the present study was consistent with that seen in phase I trials in healthy volunteers, and in overweight and obese adults.[18,26,27] There was a mild increase in serum ALT levels in all the volixibat groups compared with the placebo group, as reported in a phase I trial.[27] These elevated ALT levels were asymptomatic and were not dose-dependent in either the phase I trial or the current study. Elevated ALT levels may result from increased hepatic cholesterol turnover[38] and represent a transient and expected benign side effect of volixibat treatment. Most TEAEs were mild or moderate in severity, and serious TEAEs were reported at the same rate in the volixibat and placebo groups. No TEAEs had a fatal outcome. The most frequent TEAE in participants receiving volixibat was diarrhoea. Diarrhoea TEAEs result from increased BA concentrations in the colon, which stimulate colon motility and secretion of mucus and water, decreasing colonic transit time.[39,40] Prevalence of diarrhoea was not dose-dependent, and the incidence decreased after week 2. Intermittent diarrhoea was observed more frequently than persistent diarrhoea. Diarrhoea TEAEs were observed at similar or higher rates to those reported in phase I studies, although it should be noted that the longest treatment time in these studies was 28 days.[18,26,27]

Strengths of the present study include the predefined interim analysis, with application of a Bayesian stopping rule to determine dose selection, and the use of appropriate efficacy outcomes. In particular, the MRI-PDFF method used to assess steatosis has been shown to be more sensitive than histology in quantifying change in liver fat in patients with NASH.[41] However, it should be noted that, although the non-invasive efficacy endpoints of MRI-PDFF and serum ALT levels give an indication of steatosis, they may not fully

Table 4. TEAEs by treatment group (safety set; N = 196).

	Placebo (n = 49), n (%); m	Volixibat			
		5 mg (n = 49), n (%); m	10 mg (n = 49), n (%); m	20 mg (n = 49), n (%); m	All doses (n = 147), n (%); m
Any TEAE	37 (75.5); 130	44 (89.8); 151	44 (89.8); 171	42 (85.7); 190	130 (88.4); 512
Serious TEAE	1 (2.0); 3	1 (2.0); 1	2 (4.1); 2	0	3 (2.0); 3
Severe TEAE	2 (4.1); 4	1 (2.0); 1	4 (8.2); 6	4 (8.2); 6	9 (6.1); 13
TEAE related to IP	15 (30.6); 29	40 (81.6); 69	36 (73.5); 68	35 (71.4); 74	111 (75.5); 211
Serious TEAE related to IP	0	0	0	0	0
Severe TEAE related to IP	1 (2.0); 1	0	2 (4.1); 2	4 (8.2); 5	6 (4.1); 7
TEAEs leading to death	0	0	0	0	0
TEAEs leading to IP withdrawal/discontinuation	1 (2.0); 1	9 (18.4); 9	3 (6.1); 3	8 (16.3); 8	20 (13.6); 20
Most common TEAEs[a]					
Diarrhoea	10 (20.4); 11	38 (77.6); 43	35 (71.4); 46	35 (71.4); 47	108 (73.5); 136
Abdominal pain	3 (6.1); 3	10 (20.4); 11	9 (18.4); 10	6 (12.2); 6	25 (17.0); 27
Nausea	2 (4.1); 2	5 (10.2); 6	4 (8.2); 6	7 (14.3); 7	16 (10.9); 19
Fatigue	2 (4.1); 2	3 (6.1); 4	3 (6.1); 3	3 (6.1); 3	9 (6.1); 10
Urinary tract infection	1 (2.0); 1	1 (2.0); 2	7 (14.3); 8	1 (2.0); 1	9 (6.1); 11
Vomiting	3 (6.1); 3	1 (2.0); 1	3 (6.1); 4	4 (8.2); 4	8 (5.4); 9

IP, investigational product; m, number of events; n, number of participants experiencing the event; TEAE, treatment-emergent adverse event.
[a]TEAEs occurring in at least 5% of the volixibat all doses group.

capture the additional NASH components of inflammation and ballooning. The study was powered for the primary efficacy outcome of a 48-week reduction in NAS of at least 2 points from baseline without worsening fibrosis in liver biopsies. Early termination of the study, based on non-invasive efficacy assessments, prevented the target histologic sample size from being met. *Post hoc* evaluation of the primary efficacy outcome after termination was limited by the small numbers of participants who completed 48 weeks of treatment (n = 8–11 per group). By the time the study was terminated owing to lack of efficacy in non-invasive assessments, 43 participants had already undergone invasive and unnecessary liver biopsies. This may be considered a limitation of the study that exposed participants to potential harm. The inclusion of endpoints assessing BA profiles and microbiota may have also been a valuable addition to this study, potentially providing further insight into the metabolic effects of volixibat.

In conclusion, this phase II study did not reveal any beneficial effects of volixibat on steatosis or ALT levels after 24 weeks of treatment. The increased C4 levels do indicate that inhibiting ASBT-mediated BA reuptake with volixibat leads to an increase in hepatic BA synthesis, in accordance with the hypothesized mechanism of action. The lack of effect on liver health, in conjunction with adequate target engagement, suggests that ASBT inhibition is unlikely to be an effective treatment for patients with NASH, but offers promise for the development of ASBT inhibitors in alternative therapy areas.

Abbreviations

ALT, alanine aminotransferase; ASBT, apical sodium-dependent bile acid transporter; BAs, bile acids; BMI, body mass index; C4, 7-alpha-hydroxy-4-cholesten-3-one; IP, investigational product; LDL, low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; PCIE, probability of a clinically important effect; TEAE, treatment-emergent adverse events; ULN, upper limit of normal.

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Conflict of interest

PNN reports grants from Boehringer Ingelheim and Novo Nordisk and consultancy work (for the University of Birmingham) for Boehringer Ingelheim, Gilead Sciences Inc., Intercept Pharmaceuticals, Novo Nordisk, Pfizer, Poxel SA and Shire International. MP was an employee of Shire International (a Takeda company) during the conduct of the study. BF reports support from Shire International (a Takeda company). AS reports advisory board fees from Novartis and grants from Allergan, Cirius Therapeutics, Conatus Pharmaceuticals Inc., GENFIT, Gilead Sciences Inc., Intercept Pharmaceuticals, Madrigal Pharmaceuticals, Novartis, Novo Nordisk and Zydus Cadila. TH reports grants from Shire International (a Takeda company). H-ML was an employee of Shire International (a Takeda company) during the conduct of the study. MYS, HS, RH, PM, ZK and GPA have nothing to disclose.

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

Authors' contributions

PNN, GPA and MP contributed to the design of the study while acting as the principal coordinating investigator, the UK coordinating investigator and the sponsor's study physician, respectively. Statistical input was provided by H-ML.

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Supplementary data

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