ORIGINAL ARTICLE

Setanaxib, a first-in-class selective NADPH oxidase 1/4 inhibitor for primary biliary cholangitis: A randomized, placebo-controlled, phase 2 trial

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Abstract

Background: Primary biliary cholangitis (PBC) is a rare liver disease with significant unmet need for second-line/add-on treatments. Setanaxib, a NOX1/4 inhibitor, has shown anti-fibrotic effects in *in vitro* and animal studies. This phase 2, randomized, multicentre study investigated the efficacy and safety of setanaxib in patients with PBC.

Methods: Patients with ≥6 months of ursodeoxycholic acid (UDCA) treatment were randomized 1:1:1 to oral setanaxib 400 mg once daily (OD), twice daily (BID), or placebo, in addition to UDCA for 24 weeks. Other inclusion criteria included alkaline phosphatase (ALP) ≥1.5×ULN and gamma-glutamyl transferase (GGT) ≥1.5×ULN. The primary endpoint was percentage change from baseline in GGT at Week 24; secondary endpoints included change from baseline in ALP, liver stiffness (LS; via transient elastography), fatigue at Week 24, and safety outcomes. p values compare setanaxib 400 mg BID and placebo groups.

Results: Of patients randomized (setanaxib 400 mg OD and BID: 38, and 36; placebo: 37), 104/111 completed Week 24. Mean (standard deviation [SD]) change in GGT to Week 24 was −4.9% (59.6%) for setanaxib 400 mg OD, −19.0% (28.9%) for setanaxib 400 mg BID, and −8.4% (21.5%) for placebo; $p = .31$. Patients treated with setanaxib 400 mg OD and BID showed decreased serum ALP levels from baseline to Week 24 ($p = .002$: setanaxib BID versus placebo). Patients treated with setanaxib 400 mg OD and BID showed mean (SD) percentage increases in LS to Week 24 of 3.3% (35.0%) and 7.9% (43.7%), versus 10.1% (33.1%) for placebo ($p = .65$). Changes in mean (SD)

PBC-40 fatigue domain scores to Week 24 were +0.3% (24.9%) for setanaxib 400 mg OD, −9.9% (19.8%) for setanaxib 400 mg BID and +2.4% (23.1%) for placebo, $p = .027$. Two patients (one placebo, one setanaxib 400 mg BID) experienced serious treatment-emergent adverse events, deemed unrelated to study drug.

Conclusions: The primary endpoint was not met. However, the secondary endpoints provide preliminary evidence for potential anti-cholestatic and anti-fibrotic effects in PBC, supporting the further evaluation of setanaxib in a future phase 2b/3 trial.

KEYWORDS

cholestasis, fibrosis, NADPH oxidase 1/4 inhibitor, primary biliary cholangitis, setanaxib

1 | INTRODUCTION

Primary biliary cholangitis (PBC) is a chronic, cholestatic liver disease caused by progressive autoimmune destruction of bile ducts within the liver and the accumulation of bile acids.[1,2] Diagnosis of PBC typically relies on serum tests of liver biochemical parameters, including alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), aspartate transaminase (AST), and bilirubin, which are all typically elevated in PBC.[1] Alanine transaminase (ALT) levels may also be elevated due to inflammation.[3,4] Advanced disease progression leads to the development of liver fibrosis and eventually cirrhosis, which can be assessed through liver biopsy, or non-invasively using transient elastography.[1,4,5]

Ursodeoxycholic acid (UDCA) is the current standard of care for patients with PBC.[4] However, up to 40% of patients with PBC have an incomplete response to UDCA and may benefit from add-on or second-line therapies.[6] Biochemical response to UDCA is defined by reduced serum levels of ALP, GGT or bilirubin.[7] Elevated ALP and GGT serum levels have been shown to predict patient outcomes, including liver transplantation and death,[8,9] so incomplete response to UDCA treatment can indicate worsened disease outcomes.[9] High serum GGT levels (≥3.2×upper level of normal [ULN]) also indicate a need for treatment escalation in patients with low levels of serum ALP (<1.5×ULN).[9]

To date, there is only one second-line treatment (obeticholic acid [OCA]) approved for the treatment of PBC in UDCA incomplete responders, used in combination with UDCA.[6] In both randomized controlled clinical trials and real-world studies, OCA has shown efficacy in lowering ALP and bilirubin in ~50% of patients (according to POISE study response criteria [ALP <1.67×ULN]).[10,11] Although there is evidence that UDCA may delay histological progression in some patients with PBC, only preliminary data exist to support OCA having an anti-fibrotic effect.[12,13] Fibrates, including bezafibrate, may also be used as an add-on treatment in patients with incomplete response or intolerance to UDCA and have been shown to reduce serum ALP levels over 12 months.[14] However, at this time, bezafibrate is currently unlicensed for use in PBC.[15]

Given that liver fibrosis is a major driver of clinical outcomes in liver disease, and strong correlations between advanced fibrosis and

Lay summary

Primary biliary cholangitis (PBC) is a liver disease caused by the immune system attacking the liver. Setanaxib is a new drug being investigated to treat PBC in this initial study. Whilst the main aim of the study was not achieved, other results showed that setanaxib was well-tolerated by patients and may improve liver scarring and fatigue, supporting further testing of the potential for setanaxib to treat PBC.

liver stiffness have been previously observed,[16] improving or stabilizing liver stiffness is an important treatment target and endpoint. UDCA has been shown to stabilize or slow liver fibrosis in a subset of patients, i.e., those with a remarkable improvement in ALP, but on the other hand, many patients with incomplete response to UDCA remain at risk of progression to cirrhosis and liver failure, which can ultimately result in liver transplantation or death.[17,18]

The most commonly reported symptoms of PBC are fatigue and pruritus (itching), which can be debilitating for patients and impair quality of life (QoL).[1] While treatments such as bezafibrate have shown beneficial effects on pruritus, neither UDCA, OCA nor bezafibrate have shown efficacy in significantly reducing fatigue, despite this symptom affecting >50% of patients.[19–21]

Setanaxib, a selective inhibitor of the NADPH oxidase (NOX) 1 and 4 isoforms, has demonstrated potential to downregulate multiple fibrogenic and inflammatory pathways and prevent progression to liver fibrosis in *in vitro* and animal studies.[22,23] The mechanism of action of setanaxib involves both anti-fibrotic and anti-inflammatory effects at the early stages of pathophysiology of some liver disorders, supported by pre-clinical data.[24] These properties make setanaxib a plausible agent to evaluate. In this phase 2 clinical trial, the efficacy and safety of oral setanaxib (400 mg once [OD] or twice [BID] daily), alongside UDCA, were assessed in patients with PBC.
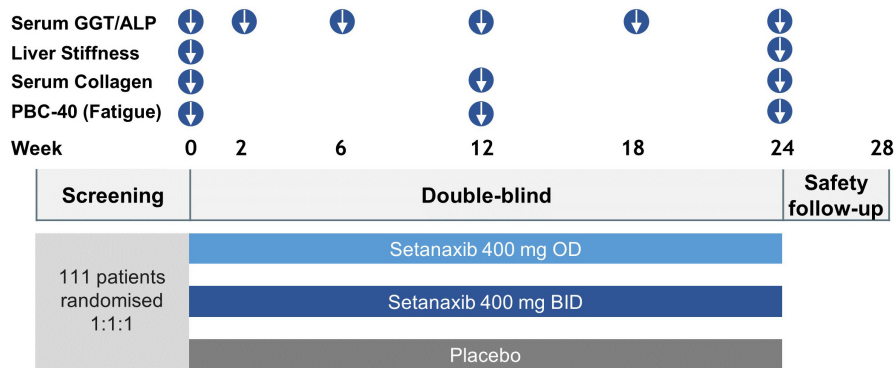
FIGURE 1 Study design.
Liver stiffness assessments were performed at baseline and Week 24 using vibration-controlled transient elastography at study sites with available equipment. PBC-40 questionnaires were self-completed at baseline and Weeks 12 and 24 by patients. All patients received setanaxib 400 mg BID or OD, or placebo, in addition to UDCA. ALP, alkaline phosphatase; BID: twice daily; GGT, gamma-glutamyl transferase; OD, once daily; UDCA, ursodeoxycholic acid.

2 | METHODS

2.1 | Study design

This double-blind, randomized, placebo-controlled, multicentre, phase 2 trial evaluated the efficacy of oral setanaxib 400 mg OD or BID, in addition to standard of care (UDCA), versus placebo, in patients with PBC and persistently elevated ALP. Patients were recruited at 61 sites (medical centres, hospitals, and universities) in nine countries (Belgium, Canada, Germany, Greece, Israel, Italy, Spain, the UK, and the US). This study was performed in accordance with the provisions of the Declaration of Helsinki, and all revisions thereof, and in accordance with USA Food and Drug Administration (FDA) regulations. Ethics approval was granted by the relevant central and regional ethics committees (Appendix 1). The trial was conducted in agreement with the International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice (GCP). This trial is registered with ClinicalTrials.gov (NCT03226067; status: completed).

2.2 | Patients

Patient eligibility was assessed during a 4-week screening period. Eligible patients were aged 18–80 years with a diagnosis of PBC, demonstrated by fulfilling ≥2 of the following diagnostic criteria at screening: (1) history of elevated ALP (>ULN) for ≥6 months, (2) a positive anti-mitochondrial antibody (AMA) titre (or PBC-specific antibodies if the AMA titre was <1:80 or negative) or (3) a historic liver biopsy consistent with PBC. Entry criteria were ALP ≥1.5×ULN (ULN: 125 U/L or 149 U/L for patients aged 19–59 or ≥60 years) and GGT ≥1.5×ULN (ULN: 65 U/L for patients aged ≥19 years).

Patients were required to have taken UDCA for ≥6 months, with a stable dose for ≥3 months, prior to their first visit; no minimum or maximum UDCA dose was specified. Those treated with cholestyramine for pruritus had to be on a stable dose for ≥8 weeks prior to their first visit and were willing to take the medication ≥2 hours before/after study medication. Patients were excluded if they were pregnant or breastfeeding, had any hepatic decompensation, planned to take or were receiving plasmapheresis or other extra-corporeal treatments for refractory pruritus or had a history of liver transplantation. Patients were excluded if they had an international normalized ratio >1.2 (except if taking anticoagulant therapy), alanine aminotransferase >3×ULN or total bilirubin >1×ULN. A list of prohibited medications is provided in Appendix 2; patients were ineligible if they had received any of these medications within 3 months of their first visit. Furthermore, patients with competing aetiologies for liver disease, such as non-alcoholic steatohepatitis, were excluded. All patients provided written informed consent to participate in the study prior to screening.

2.3 | Randomization and masking

Eligible patients were randomized 1:1:1 to setanaxib 400 mg OD, setanaxib 400 mg BID or placebo, alongside UDCA, for 24 weeks (Figure 1). Patients were stratified at study entry by disease severity (defined as baseline serum GGT <2.5×ULN or ≥2.5×ULN). To ensure balanced treatment groups, an interactive web-based randomization system was used to assign a unique randomization number in ascending, sequential order (with associated treatment arm) to each patient, based on pre-determined blocks of randomization numbers for each stratification level. The investigator was responsible for enrolling patients and assigning a randomized number (entered in their electronic case report form).

The sponsor, patients, investigators, the investigator's staff, persons performing assessments, data reviewers, and statisticians all remained blinded to the identity of the study treatments, concealed using investigational medicinal products which were identical

in packaging, labelling, administration schedule, appearance, and odour. Patients self-administered twice daily: four active capsules and four placebo capsules for the setanaxib 400 mg OD group and eight active or eight placebo capsules for the setanaxib 400 mg BID and placebo groups, respectively. Any unblinding procedures were reported by a principal investigator in the electronic case report system; none were reported. Randomization data were kept strictly confidential and were only accessible to authorized personnel until the unblinding of the trial.

2.4 | Procedures

Patients self-administered setanaxib 400 mg OD or BID or placebo orally for a total of 24 weeks. Baseline assessments were performed on Day 1; patients were then assessed on Weeks 2, 6, 12, 18, and 24. An interim analysis was conducted when 80%–90% of the planned number of randomized patients had completed Week 6; these analyses did not impact future study conduct and the results were not disclosed to trial investigators. Patients were followed up to 28 days after the end of treatment. If treatment was discontinued early, an early termination visit was scheduled, ensuring patients had six post-baseline visits.

Non-invasive measures of fibrosis, such as liver stiffness and serum levels of collagen fragments indicative of collagen formation and degradation (PRO-C3, PRO-C5, C3M, C4M, and BGM) were assessed at baseline and Week 24 (Week 12 for collagen fragments). Liver stiffness was assessed using vibration-controlled transient elastography (Fibroscan® [Echosens, Paris, France] or similar machines) by trained physicians, technicians, or radiologists at any study sites where equipment was available. The study was designed to include careful monitoring of patients, as well as being overseen by a Safety Monitoring Board to ensure patient safety.

2.5 | Outcomes

The primary efficacy endpoint, assessed by a central laboratory, was percentage change from baseline to Week 24 in serum GGT. Serum GGT was selected as the primary endpoint for this study due to its use as a marker of inflammation and oxidative stress, and the proposed anti-inflammatory and anti-fibrotic mechanism of setanaxib. We also report the following main secondary efficacy endpoints: percentage change in serum ALP, high-sensitivity C-reactive protein (hsCRP), fibrinogen, ELF, FIB-4 and APRI score, total and conjugated bilirubin, liver stiffness, serum levels of collagen fragments, and percentage change in fatigue score (based on the PBC-40 questionnaire) to Week 24.[24,25] In addition, changes in serum GGT to Week 6 are also summarized. A full list of all study outcomes, including all 17 secondary endpoints, is available in Appendix 3. To measure fatigue, patients answered 11 fatigue-based questions on the PBC-40 questionnaire using a 5-point Likert scale (highest score = highest impact) and points were summed to provide a total score.[25] The PBC-40

questionnaire was chosen to measure fatigue because it represents a patient-derived, disease-specific method of tracking fatigue symptoms, relevant to patients from varying geographical areas.[25]

Safety outcomes were monitored from baseline to the end of the follow-up period (28 days after last treatment administration). Adverse events (AEs) were coded using MedDRA™ version 21.1 and graded according to the common terminology criteria for AEs (CTCAE) Version 4. We report treatment-emergent AEs (TEAEs; defined as any AE that started on/after the date of first treatment dose), related TEAEs, serious TEAEs, severe TEAEs (defined as any AE grade 3 or above using the CTCAE), TEAEs leading to discontinuation or interruption of study treatment, and AEs leading to death.

2.6 | Statistical analysis

A sample size of 34 patients within each treatment group (a total of 102 patients) was required to have 80% power to detect a 28% difference in mean percentage changes from baseline in serum GGT between treatment groups. A Wilcoxon Mann–Whitney test was used for this sample size estimate; this was the most conservative approach for statistical power. Standard deviations (SD) of 30 for the setanaxib groups and 40 for the placebo group were assumed based on a recent phase 3 clinical trial of OCA.[26] An overall two-sided Type I error of 5% was assumed and the Hochberg method was used to adjust the alpha level for the two-dose comparisons of percentage change in serum GGT and ALP at Week 24; setanaxib 400 mg BID and setanaxib 400 mg OD dose levels were tested against alpha levels of 0.047 and 0.023, respectively. This step-down procedure, after accounting for correlation, ensured that the overall alpha level was no more than 5%. No further multiplicity adjustments were made for the comparison of other efficacy endpoints. Demographic data were compared for setanaxib 400 mg OD and setanaxib 400 mg BID versus placebo using an Analysis of Variance (ANOVA) or a Mann–Whitney test for continuous variables as appropriate, and Fisher's exact test for categorical variables.

The primary endpoint was analysed using the intention-to-treat population (all randomized patients). The percentage change in serum GGT from baseline to Week 24 was analysed using an Analysis of Covariance (ANCOVA) with treatment and disease severity as fixed effects, and baseline GGT as a continuous covariate. The estimated difference between the setanaxib 400 mg BID group and placebo was calculated, along with 95% confidence intervals (CI). Reported p values compare setanaxib 400 mg BID and placebo groups. In the event of missing data at Week 24, the last observation carried forward (LOCF) method was applied, by imputing Week 24 visit data with the last non-missing post-baseline data available from Week 12 onwards, where Week 12 data were collected. A secondary analysis of GGT including all post-baseline visits over the 24-week treatment period (assessments at Weeks 2, 6, 12, 18, and 24) was performed using a repeated measures ANOVA with treatment, visit and disease severity as fixed effects and baseline GGT as a continuous covariate plus an interaction term for treatment and visit. With the inclusion of longitudinal data from the same subjects within each arm, variability

is reduced and statistical power is increased when compared with an ANCOVA that compares data only at Week 24.

The secondary endpoint of percentage change in serum ALP was analysed using the same statistical methods as the primary endpoint. No significant tests were performed for other secondary liver disease outcomes. For change in fatigue score from baseline to Week 24, treatment differences and associated 95% CI are presented with p values to compare setanaxib 400 mg BID and placebo treatment groups. Data were summarized descriptively. For pre-planned subgroup analyses, patients were grouped at baseline according to a pre-defined liver stiffness cut-off value of 9.6 kilopascals (kPa), as recommended by the European Association for the Study of the Liver (EASL) Clinical Practice Guidelines.[4] These cut-offs were chosen due to a previous study reporting that patients with liver stiffness >9.6 kPa have a five-fold increased risk of PBC-related complications, indicating a higher risk patient group with greater unmet need.[17] Post-hoc analyses were conducted to explore the effect of treatment in subgroups according to baseline liver stiffness (<9.6 kPa and ≥9.6 kPa). The safety population included all patients who received ≥1 dose of treatment and had ≥1 safety assessment. Safety data were periodically reviewed by the Safety Monitoring Committee. All analyses were performed using SAS Version 9.4.

3 | RESULTS

3.1 | Baseline characteristics

A total of 189 patients were screened, of whom 111 were randomized between 4th October 2017 and 25th September 2018 to either setanaxib 400 mg OD (n = 38), setanaxib 400 mg BID (n = 36) or placebo (n = 37; Figure S1). Among the screening failures, most failed the serum ALP (n = 40) or GGT (n = 33) inclusion criteria (≥1.5 × ULN); many screened patients failed multiple inclusion/exclusion criteria. After 24 weeks, 104 patients completed treatment. Seven patients discontinued treatment from setanaxib 400 mg OD (n = 3) and BID (n = 4). Four patients discontinued setanaxib due to TEAEs (increased GGT, hypersensitivity, abdominal distension, dyspnoea, palpitations, and increased transaminases; listed by MedDRA preferred term, Table 2). Three further patients discontinued due to change in UDCA dose (n = 1), withdrawal of consent (n = 1) or administrative reasons (n = 1; Figure S1). The study ended after the last patient attended their Week 28 follow-up visit.

Baseline characteristics were similar between treatment groups, although patients randomized to setanaxib 400 mg BID had higher serum ALP (p = .046) and ALT (p = .042) levels than placebo (Table 1). Those randomized to setanaxib (400 mg OD and BID) had been diagnosed approximately 5 years prior to the placebo group, though the difference in time since first PBC diagnosis was only significant for patients randomized to setanaxib 400 mg OD (p = .049). Patients randomized to setanaxib 400 mg OD had a higher median baseline UDCA dose (mg/kg/day) than placebo (p = .017), although there was no significant difference between patients randomized to setanaxib

400 mg BID versus placebo (p = .085). No significant differences were found between the setanaxib 400 mg OD and setanaxib 400 mg BID treatment groups compared with placebo for other baseline characteristics in Table 1. For all treatment groups, patients were predominantly female (n = 99; 89.2%) and mean age was 56.2 years, in line with reported demographic characteristics of patients with PBC.[5] Median baseline UDCA doses were 14.6, 14.9, and 13.4 mg/kg/day for setanaxib 400 mg OD, setanaxib 400 mg BID and placebo, respectively (Table 1; doses ranged from 3–68 mg/kg/day). Using vibration-controlled transient elastography, 91 patients (82.0%) had valid liver stiffness measurements at baseline and Week 24. Patients in the placebo group had higher median baseline liver stiffness than in the setanaxib 400 mg OD and BID groups (8.9 kPa versus 8.0 kPa, and 7.5 kPa, respectively); however, the differences were not statistically significant. Mean fatigue scores at baseline were generally comparable across treatment groups. Baseline characteristics for liver stiffness subgroups (<9.6/≥9.6 kPa) are available in Table S1.

3.2 | Changes in liver biochemistry

Patients receiving setanaxib 400 mg BID showed a mean (SD) −22.0% (23.4%) change from baseline to Week 6 in serum GGT, with a change of −11.8% (21.6%) for setanaxib 400 mg OD patients and −7.5% (16.9%) for placebo-treated patients. At Week 6, a statistically significant difference was observed in the mean change in serum GGT between patients treated with setanaxib 400 mg BID and placebo (p = .0057). Mean (SD) reductions from baseline to Week 24 were −4.9% (59.6%) and −19.0% (28.9%) for setanaxib 400 mg OD and BID and −8.4% (21.5%) for placebo; the difference between setanaxib 400 mg BID and placebo was −9.7% (95% CI −28.5% to 9.0%). The primary endpoint of percentage change in serum GGT from baseline to Week 24 did not meet statistical significance for setanaxib 400 mg BID versus placebo (p = .31; Figure 2A).

Patients treated with setanaxib 400 mg OD and BID experienced greater reductions in mean (SD) serum ALP from baseline to Week 24 (OD: −9.7% [21.1%]; BID: −12.9% [19.6%]) versus placebo (−3.1% [16.0%]); the difference between setanaxib 400 mg BID and placebo was −9.0% (95% CI −17.9% to 0.0%; Figure 2B). At Week 24, the difference between setanaxib 400 mg BID and placebo (p = .049; significance level 0.047) did not reach statistical significance after adjustment for multiple comparisons. However, over the full 24-week treatment period (repeated measures analysis), the difference between setanaxib 400 mg BID and placebo was statistically significant (p = .002). For both serum GGT and ALP, the difference in mean percentage change from baseline between the setanaxib 400 mg BID and placebo treatment groups was most pronounced at Week 6. Median absolute change from baseline data for GGT and ALP is reported in Table S2.

After post-hoc stratification by baseline liver stiffness, mean (SD) percentage change in serum GGT from baseline to Week 24 for setanaxib 400 mg OD and BID versus placebo was +9.1% (90.8%) and −32.4% (20.8%) versus −10.3% (23.6%), respectively, in patients with liver stiffness ≥9.6 kPa (n = 39; Figure 3A); however, the difference

TABLE 1 Patient baseline demographics and disease characteristics.

	Placebo (n = 37)	Setanaxib 400 mg OD (n = 38)	Setanaxib 400 mg BID (n = 36)
Age (years), mean (SD)	56.3 (9.2)	56.8 (9.1)	55.6 (9.0)
Female, n (%)	35 (94.6)	30 (78.9)	34 (94.4)
Racial group: Caucasian, n (%)	36 (97.3)	38 (100.0)	36 (100.0)
BMI (kg/m^2), mean (SD)	27.6 (5.3)	27.2 (4.1)	26.4 (6.1)
Time since first PBC diagnosis (months), median (min, max)	129.1 (11.0, 317.0)	62.4 (1.0, 391.0)*	70.1 (7.0, 326.0)
Age at PBC diagnosis (years), mean (SD)	46.2 (8.5)	48.9 (9.1)	48.1 (9.1)
Time since start of UDCA treatment (months), mean (SD)	81.4 (79.0)	81.5 (90.6)	68.5 (67.3)
Serum GGT (U/L), median (IQR)	169.0 (113.0–260.0)	189.0 (121.0–351.0)	194.0 (143.5–263.0)
Serum ALP (U/L), median (IQR)	251.0 (217.0–298.0)	267.0 (227.0–334.0)	319.5 (242.5–400.5)*
Total serum bilirubin (μmol/L), median (IQR)	10.0 (7.0–13.0)	10.5 (8.0–14.0)	10.0 (7.0–13.0)
hsCRP (mg/L), mean (SD)	4.8 (4.6)	5.8 (5.2)	5.1 (5.1)
Liver stiffness (kPa), median (IQR)[a]	8.9 (6.6)	8.0 (6.6)	7.5 (6.3)
Baseline fatigue, mean (SD)	28.4 (12.1)	26.7 (10.7)	29.5 (11.3)
Baseline UDCA dose (mg/kg/day), median (min, max)	13.4 (3.0, 23.0)	14.6 (9.0, 34.0)*	14.9 (3.0, 68.0)
Bile acids (μmol/L), median (min, max)	27.4 (6.2, 73.2)	18.7 (4.9, 160.1)	17.7 (4.7, 102.3)
Fibrinogen (g/L), mean (SD)	3.7 (0.8)	3.7 (0.7)	3.8 (0.7)
FIB-4 score, mean (SD)	2.0 (1.2)	2.1 (1.5)	1.8 (1.0)
APRI score, mean (SD)	0.5 (0.3)	0.6 (0.4)	0.6 (0.4)
ELF score, mean (SD)	9.7 (1.0)	10.2 (1.1)	9.7 (1.0)
Serum AST (U/L), mean (SD)	43.0 (17.4)	43.6 (20.5)	50.0 (31.3)
Serum ALT (U/L), mean (SD)	42.9 (16.5)	44.7 (22.0)	56.2 (35.3)*
Conjugated bilirubin (μmol/L), mean (SD)	6.0 (2.1)	6.1 (2.3)	6.4 (2.4)

Note: Baseline demographics and disease characteristics reflect the intention-to-treat population. Asterisks indicate a significant difference at baseline compared with placebo (*$p < .05$).

[a]For baseline liver stiffness (values are included for patients who provided measurements at Week 0 and Week 24), placebo: n = 32; setanaxib 400 mg OD: n = 33; setanaxib 400 mg BID: n = 26.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; APRI, AST to platelet ratio index; AST, aspartate transaminase; BID, twice daily; BMI, body mass index; ELF, enhanced liver fibrosis; FIB-4, fibrosis-4; GGT, gamma-glutamyl transferase; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; L, litres; kPa, kilopascal; OD, once daily; PBC, primary biliary cholangitis; U, units; UDCA, ursodeoxycholic acid; SD, standard deviation.

between setanaxib 400 mg BID and placebo was not statistically significant ($p = .33$). Similarly, in patients with liver stiffness <9.6 kPa (n = 56; Figure 3A), the difference in mean percentage change in serum GGT from baseline to Week 24 for setanaxib 400 mg BID (−12.9% [29.7%]) versus placebo (−5.4% [20.4%]) was not statistically significant ($p = .39$). A statistically significant difference was observed between mean (SD) percentage change in serum ALP from baseline to Week 24 for setanaxib 400 mg BID (−24.3% [12.4%]) versus placebo (−3.3% [16.9%]) in patients with liver stiffness ≥9.6 kPa (Figure 3B; $p = .024$). In patients with liver stiffness <9.6 kPa, the corresponding mean percentage changes were not statistically significant at −8.7% (20.0%) versus −1.8% (16.0%), respectively (Figure 3B; $p = .27$). By Week 24, all treatment groups showed an increase from baseline in total bile acids, with median (minimum, maximum) percentage changes of +9.9% (−67.7%, 408.7%) for setanaxib 400 mg OD, +26.3% (−63.5%, 1822.0%) for setanaxib 400 mg BID and +0.6% (−86.3%, 190.6%) for placebo. For changes in hsCRP, patients

randomized to setanaxib 400 mg OD, setanaxib 400 mg BID, and placebo experienced mean (SD) percentage increases of 11.7%, 5.7%, and 8.0%, respectively. Mean percentage changes from baseline to Week 24 in fibrinogen, FIB-4, APRI and ELF scores, serum AST and ALT and conjugated and total bilirubin are shown in Table S3. No p values were calculated for these secondary outcomes.

3.3 | Changes in liver stiffness and markers of fibrosis

In total, 91 patients had available liver stiffness readings at baseline and Week 24. Of these patients, those treated with setanaxib 400 mg OD or BID experienced +0.1 kPa and −0.4 kPa absolute changes in median liver stiffness, respectively, compared to +0.4 kPa for placebo. Median absolute liver stiffness at baseline and Week 24 for each treatment group is presented in Figure S2A.

**FIGURE 2** Mean percentage change in serum GGT and ALP over 24 weeks of treatment.

Intention-to-treat population. *p* values compare setanaxib 400 mg BID (in addition to UDCA) and placebo (in addition to UDCA) treatment groups. When considering the whole 24-week treatment period, *p* = .12 for GGT, and *p* = .002 for ALP. *Close to statistical significance after adjustment for multiple comparisons. Error bars indicate SD. ALP, alkaline phosphatase; BID, twice daily; GGT, gamma-glutamyl transferase; OD, once daily; SD, standard deviation.

(A) Serum GGT
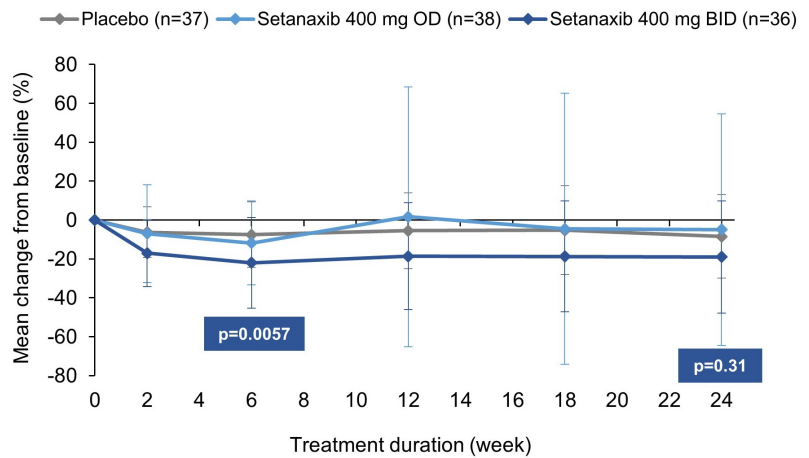
Placebo	−6.2	−7.5	−5.5	−5.2	−8.4
Setanaxib 400 mg OD	−7.0	−11.8	1.7	−4.5	−4.9
Setanaxib 400 mg BID	−17.0	−22.0	−18.6	−18.7	−19.0

(B) Serum ALP
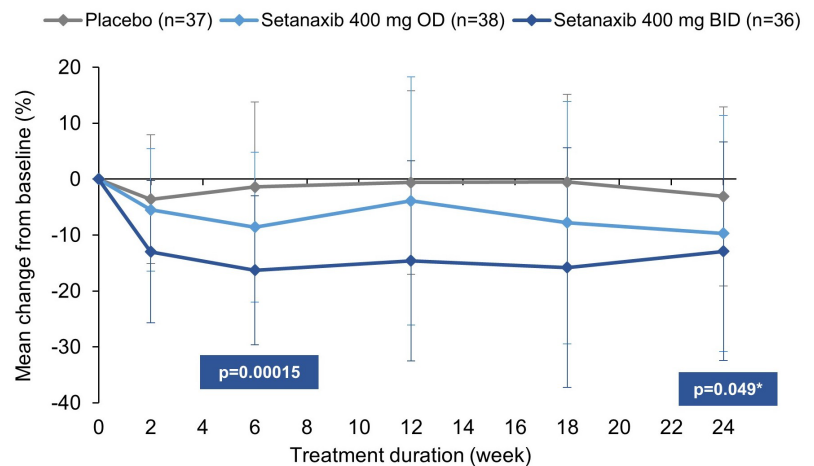
Placebo	−3.6	−1.4	−0.6	−0.5	−3.1
Setanaxib 400 mg OD	−5.5	−8.6	−3.9	−7.8	−9.7
Setanaxib 400 mg BID	−13.0	−16.3	−14.6	−15.8	−12.9

After *post-hoc* stratification by baseline liver stiffness, patients with baseline liver stiffness ≥9.6 kPa treated with setanaxib 400 mg OD (*n* = 14) and BID (*n* = 9) experienced absolute reductions in median liver stiffness by Week 24 of −1.0 kPa and −3.0 kPa, respectively, compared with −0.7 kPa for placebo (*n* = 16; Figure S2B).

Reductions in serum levels of types III (PRO-C3) and V (PRO-C5) collagen were observed after 24 weeks in all treatment groups; these reductions were more profound in the setanaxib 400 mg OD and BID groups (Figure 4). Reductions were also shown in C3M, C4M, and BGM serum levels, all indicating collagen degradation, in all three treatment groups after 24 weeks.

Median percentage changes in collagen serum levels after 24 weeks, for patients by liver stiffness subgroup, are shown in Figure S3. Patients with baseline liver stiffness ≥9.6 kPa receiving setanaxib 400 mg BID showed a 13.7% reduction in type III collagen

formation (PRO-C3) and a 15.4% increase in type III collagen degradation (C3M), while less consistent signals were shown for patients receiving setanaxib 400 mg OD and placebo, and patients with baseline liver stiffness <9.6 kPa.

3.4 | Changes in fatigue scores

Baseline fatigue scores for the three treatment groups were comparable (Table 1). At Week 24, patients treated with setanaxib 400 mg BID (*n* = 30) reported a mean (SD) percentage change in fatigue score of −9.9% (19.8%; *n* = 30; Figure 5). In comparison, minimal change from baseline to Week 24 was observed amongst patients treated with setanaxib 400 mg OD (+0.3% [24.9%]; *n* = 37), and patients receiving placebo reported a mean (SD) increased fatigue score

(A) Serum GGT
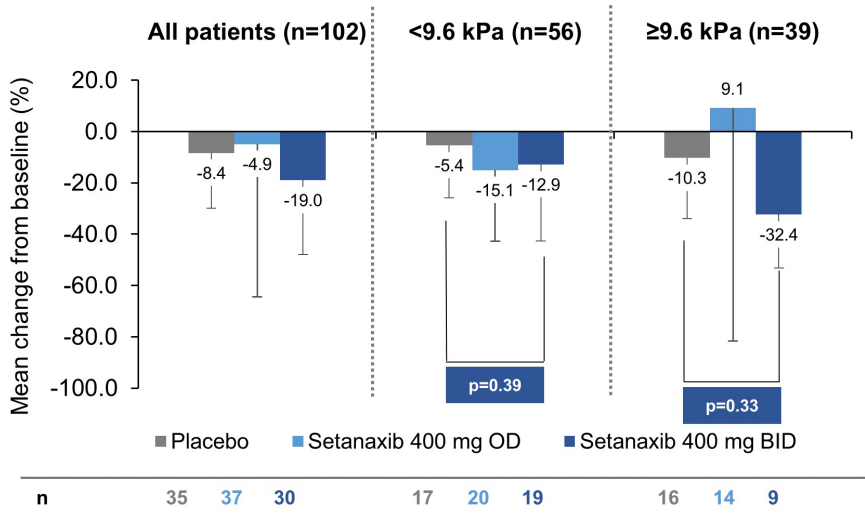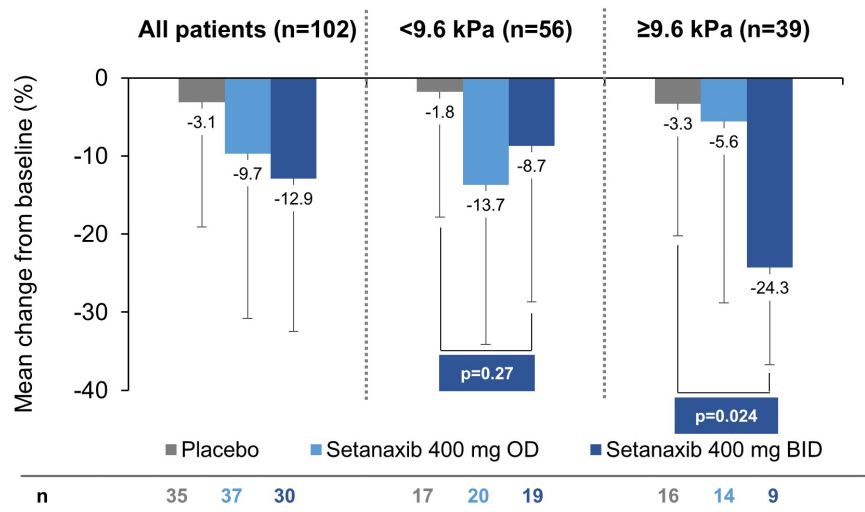

(B) Serum ALP

FIGURE 3 Mean percentage change in serum GGT and ALP between baseline and Week 24, stratified by baseline liver stiffness.

Intention-to-treat population. n numbers given underneath figure represent patients included in the analysis. Liver stiffness assessments were performed at baseline and Week 24 using vibration-controlled transient elastography at study sites with available equipment. In total, 16 patients were not included in the subgroup analysis either due to missing liver stiffness data at baseline ($n = 7$) or missing serum GGT and ALP data at Week 24 ($n = 9$). Data represent observed case. Error bars indicate SD. ALP, alkaline phosphatase; BID, twice daily; GGT, gamma-glutamyl transferase; kPa, kilopascal; OD, once daily; SD, standard deviation.

of +2.4% (23.1%; $n = 36$). The difference between mean change in fatigue scores from baseline to Week 24 for the setanaxib 400 mg BID and placebo groups was −12.2, deemed statistically significant ($p = .027$; 95% CI −22.9 to −1.4).

3.5 | Safety

By Week 24, 96 (86.5%) and 2 (1.8%) patients had experienced a TEAE and serious TEAE, respectively. TEAEs were generally balanced across treatment groups, aside from infections and infestations, which occurred in fewer patients treated with setanaxib 400 mg OD and BID versus placebo (Table 2). TEAEs were clustered in gastrointestinal disorders, infections and infestations, skin and subcutaneous tissue disorders, nervous system disorders, musculoskeletal and connective tissue disorders, and general disorders and administration site conditions system organ classes. The serious TEAEs included one case of grade 1 urinary infection requiring hospitalization (placebo) and one case of multiple bone fractures due

to a road traffic accident (setanaxib 400 mg BID), both deemed unrelated to the study drug by investigators. Overall, 3.6% of patients experienced treatment interruptions due to AEs; there were no deaths or drug-related serious AEs during the study.

4 | DISCUSSION

This phase 2 study was the first trial to investigate the effect of the first-in-class selective NOX 1/4 inhibitor, setanaxib (400 mg OD or BID), on markers of cholestasis and liver fibrosis in patients with PBC. Our results, particularly those from secondary endpoints, provide preliminary evidence for the potential anti-cholestatic, anti-inflammatory and, anti-fibrotic effects of setanaxib (400 mg OD or BID, used alongside the standard of care UDCA), as well as the reduction of fatigue.

Although the primary endpoint was not met, patients treated with setanaxib 400 mg BID showed a larger reduction in serum GGT after 24 weeks of treatment than patients receiving placebo. The mean results for serum GGT levels in patients treated with setanaxib

FIGURE 4 Median percentage change from baseline in collagen fragment serum levels after 24 weeks of treatment. Intention-to-treat population. PRO-C3 and PRO-C5 are biomarkers that indicate the formation of type III and type V collagen, respectively. Error bars indicate IQR. C3M, C4M and BGM indicate collagen III and IV and biglycan degradation. BGM, biglycan degradation; BID, twice daily; C3M: collagen III degradation; C4M, collagen IV degradation; IQR, interquartile range; OD, once daily; PRO-C3/C5, pro-peptide of type III/V collagen.
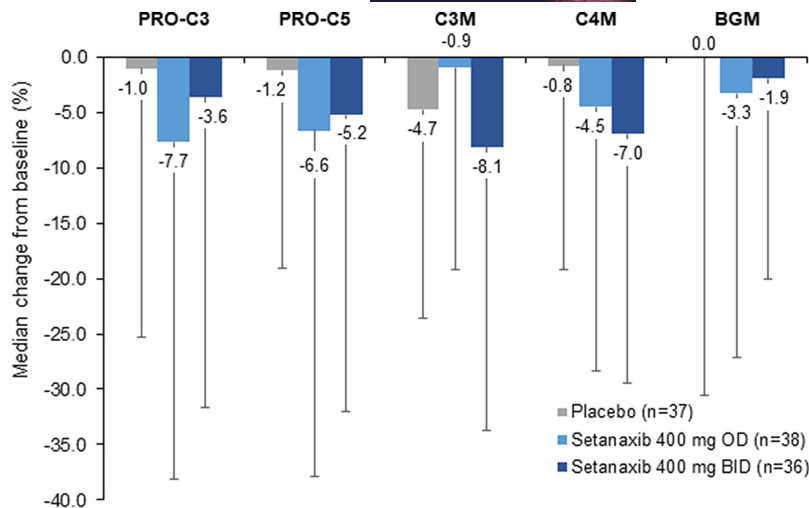
Median baseline collagen fragment serum levels (ng/ml)

	PRO-C3	PRO-C5	C3M	C4M	BGM
Placebo	15.1	970.8	15.5	51.4	15.3
Setanaxib 400 mg OD	20.8	1246.7	16.7	57.7	18.3
Setanaxib 400 mg BID	19.0	1076.7	15.1	49.0	17.3

FIGURE 5 Change in mean fatigue score from baseline after 12 and 24 weeks of treatment. Intention-to-treat population. p value compares setanaxib 400 mg BID and placebo treatment groups (both in addition to UDCA). Analysed via ANCOVA. Error bars indicate SD. ANCOVA, analysis of covariance; BID, twice daily; OD, once daily; SD, standard deviation.
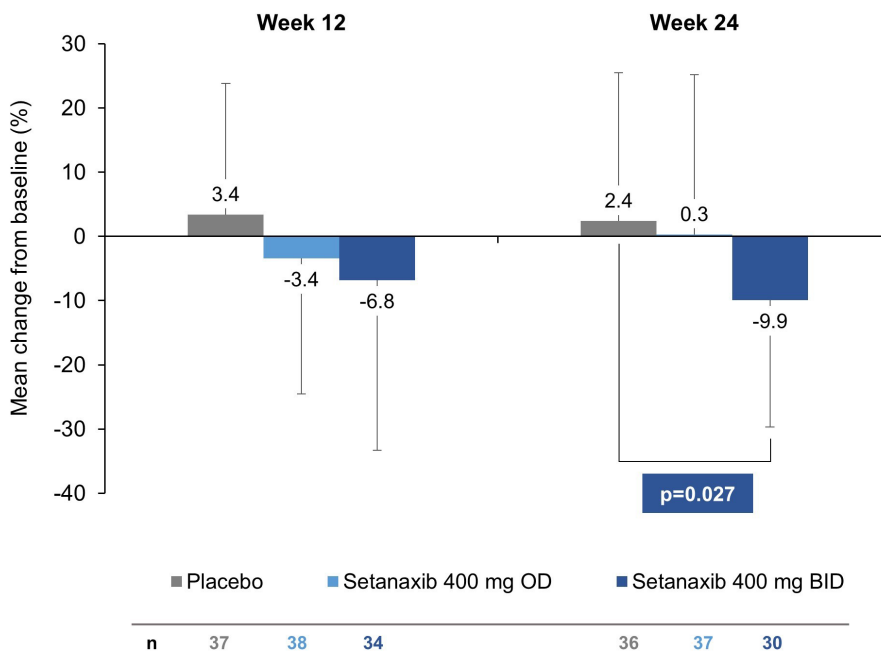
400 mg OD were affected by a single patient with a large increase in serum GGT levels, contributing to non-normal distribution of data in this group. However, this increase in serum GGT levels was considered unrelated to the setanaxib treatment by the study investigator. The difference in percentage change in serum ALP levels from baseline was close to significance at Week 24, for setanaxib 400 mg BID versus placebo, indicating a potential anti-cholestatic effect. In a secondary repeated measures analysis that included all post-baseline assessments, there was a significantly greater reduction in ALP for setanaxib 400 mg BID versus placebo. This can be attributed to reduced variability and higher statistical power of this analysis method.

When stratified by baseline liver stiffness, the largest reductions in GGT and ALP levels following 24 weeks of treatment with setanaxib 400 mg BID were observed in patients with liver stiffness ≥9.6 kPa. This suggests that setanaxib 400 mg BID treatment may have a greater effect in patients with elevated liver stiffness, who are difficult to treat with currently available first- and second-line therapies such as UDCA, which has a higher rate of treatment failure in patients with increased liver fibrosis.[5] However, the difference in change from baseline in GGT levels between patients treated with setanaxib 400 mg BID and placebo at Week 24 was not statistically significant within either the ≥9.6 kPa or <9.6 kPa liver stiffness

TABLE 2 Safety outcomes after 24 weeks of treatment.

n (%) [events]	Placebo (n = 37)	Setanaxib 400 mg OD (n = 38)	Setanaxib 400 mg BID (n = 36)
Any TEAE (by SoC)	31 (83.8) [121]	34 (89.5) [119]	31 (86.1) [100]
Gastrointestinal disorders	15 (40.5) [22]	15 (39.5) [25]	17 (47.2) [25]
Infections and infestations	15 (40.5) [24]	9 (23.7) [12]	8 (22.2) [11]
Skin and subcutaneous tissue disorders	11 (29.7) [12]	11 (28.9) [15]	10 (27.8) [14]
Nervous system disorders	8 (21.6) [12]	10 (26.3) [17]	7 (19.4) [8]
Musculoskeletal and connective tissue disorders	8 (21.6) [10]	10 (26.3) [12]	6 (16.7) [6]
General disorders and administration site conditions	8 (21.6) [14]	4 (10.5) [6]	9 (25.0) [12]
Any related TEAE	12 (32.4) [21]	16 (42.1) [24]	17 (47.2) [28]
Serious TEAEs[a]	1 (2.7) [1]	0 (0.0) [0]	1 (2.8) [1]
Severe TEAEs	1 (2.7) [3]	0 (0.0) [0]	3 (8.3) [6]
TEAEs leading to study drug discontinuation (by PT)	0 (0.0) [0]	2 (5.3) [2]	2 (5.6) [4]
GGT increased	0 (0.0) [0]	1 (2.6) [1]	0 (0.0) [0]
Transaminases increased	0 (0.0) [0]	0 (0.0) [0]	1 (2.8) [1]
Palpitations	0 (0.0) [0]	0 (0.0) [0]	1 (2.8) [1]
Abdominal distension	0 (0.0) [0]	0 (0.0) [0]	1 (2.8) [1]
Hypersensitivity	0 (0.0) [0]	1 (2.6) [1]	0 (0.0) [0]
Dyspnoea	0 (0.0) [0]	0 (0.0) [0]	1 (2.8) [1]
TEAEs leading to study drug interruption	1 (2.7) [1]	1 (2.6) [2]	2 (5.6) [2]
Non-TEAEs	5 (13.5) [6]	3 (7.9) [3]	9 (25.0) [12]
Any AE leading to death	0 (0.0) [0]	0 (0.0) [0]	0 (0.0) [0]

Note: Safety events are reported for the safety population (n = 111) according to MedDRA Version 21.1. AEs were graded using the CTCAE version 4. TEAEs by system organ class are ranked according to AE incidence; only those present in >10% of total patients are shown.

[a]These serious TEAEs include one case of grade 1 urinary infection (preferred term) requiring hospitalization (placebo group) and one case of multiple bone fractures (preferred term) due to a road traffic accident (setanaxib 400 mg BID group); both cases were deemed unrelated to the study drug by the investigators.

Abbreviations: AE, adverse event; BID, twice daily; GGT, gamma-glutamyl transferases; OD, once daily; PT, preferred term; SoC, system organ class; TEAE: treatment-emergent adverse event.

subgroup. Longer term data are required to confirm whether setanaxib treatment may have a clinically significant impact on serum GGT levels in patients with PBC and elevated liver stiffness.

Both currently approved therapies for PBC, UDCA, and OCA have been shown to significantly reduce markers of cholestasis (GGT and ALP) over time. However, nearly half of OCA-treated patients failed to respond in the POISE trial, and inadequate responders remain at high risk for disease progression. In addition, there are many other unresolved aspects of PBC significantly impacting patient outcomes and QoL that are not addressed by current treatments, including liver fibrosis and fatigue. Liver fibrosis is a strong predictor of poor long-term outcomes in PBC, with advanced-stage fibrosis often resulting in liver decompensation, requirement for liver transplantation and death.[17]

In this study, patients with elevated liver stiffness (≥9.6 kPa) treated with setanaxib 400 mg BID experienced a median absolute reduction of −3.0 kPa liver stiffness at Week 24, possibly suggesting the potential for reduced fibrosis when treated with higher doses of setanaxib in this patient group. This patient population is at higher risk of worse long-term disease outcomes than patients with lower baseline liver stiffness, thus representing patients with greater unmet need.[17,27] Although a recent open-label study of bezafibrate plus UDCA showed that 48% of patients achieved fibrosis regression after five years' treatment, to date no large-scale, randomized controlled trials have been conducted to validate this outcome.[28,29] Elafibranor and seladelpar have shown potential for reducing markers of cholestasis but have not yet shown beneficial effects on liver fibrosis in patients with PBC.[19,30] At the time of writing, these

therapies were at the early stages of investigation and not currently approved for treatment.

It should be noted that liver stiffness can also be affected by pathological processes such as inflammation and oedema, and setanaxib is also known to have anti-inflammatory properties.[24] Collagen fragment serum levels were assessed to further investigate the potential anti-fibrotic effect of setanaxib 400 mg OD or BID.[31] It was previously reported that cirrhotic livers contain approximately four-fold as much type III collagen as healthy livers (8 mg/g versus 2 mg/g wet weight in a healthy liver).[32] Our results showed reduced markers of types III and V collagen formation (PRO-C3 and PRO-C5) after 24 weeks across all three treatment groups, but this was more pronounced in the setanaxib groups versus placebo (no p value calculated). When analysed by liver stiffness subgroups, the greatest median reduction in PRO-C3 was observed in patients with liver stiffness ≥9.6 kPa at baseline receiving setanaxib 400 mg BID, which was accompanied by an increase in serum levels of C3M (no p value calculated). Given that these patients also had advanced fibrogenesis at baseline, as indicated by median baseline PRO-C3 levels of >20 ng/mL, this finding is in line with previous results from a phase 2 clinical trial in primary sclerosing cholangitis.[33] Together, these results may support the theory that in patients with clinically significant fibrogenesis at baseline, the improvements in liver stiffness observed after setanaxib 400 mg BID treatment may be partly due to fibrosis reduction, either with or without additional reduction of inflammation. However, due to small patient numbers in this subgroup ($n = 14$), these results should be interpreted with caution and further research is warranted to investigate the potential anti-fibrotic effect of setanaxib.

Chronic fatigue is one of the most common symptoms associated with PBC and is highly prioritized by patients as a treatment target, due to its debilitating effects on QoL and limited treatment options.[19] Our results suggest that setanaxib 400 mg BID can produce clinically significant improvements (defined as a mean reduction of 0.5/item in the PBC-40 fatigue domain)[34] in symptoms of fatigue versus placebo. This represents an area of unmet need in the treatment of PBC; whilst some patients have previously reported fatigue improvements after 1 year of seladelpar treatment,[19] only preliminary results using a non-validated approach have been reported as to the effects of bezafibrate plus UDCA on fatigue.[35]

Setanaxib 400 mg OD and BID were well-tolerated during 24 weeks of treatment, with only four patients experiencing treatment interruptions (pausing and resuming of study drug during the study) due to TEAEs and four patients discontinuing treatment due to TEAEs. This safety profile supports the use of setanaxib 400 mg OD or BID alongside generically available anti-cholestatic agents, such as UDCA, and is in line with other studies reporting only mild AEs, including loose stools and mild diarrhoea, in patients with PBC treated with UDCA alone.[36]

The impact of our findings is limited by the study being insufficiently powered for the analysis of secondary efficacy outcomes. Throughout treatment, patients received different stable concomitant doses of UDCA and had differing lengths of exposure; this could have affected study outcomes. The use of vibration-controlled transient elastography to assess liver stiffness provided a less invasive alternative compared to liver biopsy, but it is not without limitations.[37,38] Although one large multicentre study concluded that Fibroscan® provides an accurate assessment of liver fibrosis,[37] it is also accepted that liver stiffness measurements via Fibroscan® can be affected by inflammation, venous pressure, and cholestasis.[38] Thus, these early findings should be interpreted with caution. Further studies will be beneficial to fully assess the benefits of setanaxib treatment (alongside UDCA) on liver fibrosis and fatigue, as well as overall control of the disease, in patients with PBC.

This study enrolled 111 patients with PBC across nine countries, making it one of the largest and longest phase 2 trials conducted in this indication and generalizable globally. The patient population of this study was representative of the PBC clinical demographic; 82% of study participants were female (versus up to 90% of patients with PBC in clinical settings) and the average age of disease onset was 56.2 years (typically >50 years).[5] The ALP level required for eligibility in this study (≥1.5 × ULN) was previously verified by EASL as the point at which long-term risk of liver transplantation or death becomes higher than in a sex- and age-matched healthy population, confirming that this study population represents patients with active disease.[4] However, in the subgroup analyses stratified by baseline liver stiffness, the patient numbers per group were low and thus observations made in these subgroups should be confirmed in a larger patient sample in a future phase 2b/3 trial.

In conclusion, the results obtained in this 24-week, phase 2 trial suggest that setanaxib, a first-in-class selective NOX 1/4 inhibitor, maybe a novel treatment deserving further investigation for patients with PBC. When used in addition to UDCA, the preliminary evidence reported here suggests potential anti-cholestatic, anti-inflammatory and anti-fibrotic effects, and fatigue reduction. Setanaxib was shown to be well-tolerated at both doses (400 mg OD and BID). These results support the further evaluation of setanaxib in a phase 2b/3 trial in patients with PBC and elevated liver stiffness.

AUTHOR CONTRIBUTIONS

CONFLICT OF INTEREST STATEMENT

PI: Grant/research support from Bruschettini SRL, Gilead Sciences, Intercept Pharmaceuticals. MC: Consultancy fees from Calliditas Therapeutics AB, CymaBay Therapeutics, Echosens, Genkyotex, Intercept Pharmaceuticals, Mayoly Spindler, Moderna Inc., Perspectum; Grant/research support from Genetic SpA, Intercept Pharmaceuticals. DJ: Consultancy and grant funding from Intercept; Speaker fees from Abbott, Falk; Consultancy fees from Calliditas Therapeutics AB, GSK. CL: Grant/research support from Alnylam Pharmaceuticals, Calliditas Therapeutics AB, Cara Therapeutics, CymaBay Therapeutics, Genkyotex, GENFIT, Gilead Sciences, GSK, HighTide Therapeutics, Intercept Pharmaceuticals, Mirum Pharmaceuticals, Mitsubishi Tanabe Pharma Corporation, Novartis, Pliant, Zydus Pharmaceuticals; Consultancy fees from Calliditas Therapeutics AB, Cara Therapeutics, GENFIT, GSK, Mirum Pharmaceuticals, Inc., Pliant Therapeutics, Teva Pharmaceuticals. NL: Consultancy fees from Calliditas Therapeutics AB. PW: Employed at Genkyotex; stock shareholder at Genkyotex. FN: Consultancy fees from: AbbVie, AgomAb Therapeutics, Biotest, BMS, Chemomab Therapeutics, Cook Medical, Durect, Dynacure, Ferring, Fundplus, Genkyotex, Gilead, Gore, Intercept, Ipsen, Mayoly Spindler, MedPartners, MSD, Promethera, and Twin Pharma.

DATA AVAILABILITY STATEMENT

Study data, including individual participant data, will not be made available to others after publication. The study protocol is available at https://clinicaltrials.gov/ct2/show/study/NCT03226067. No additional related documents will be made available.

ETHICAL APPROVAL STATEMENT

This study was performed in accordance with the provisions of the Declaration of Helsinki, and all revisions thereof, and in accordance with USA Food and Drug Administration (FDA) regulations. Ethics approval was granted by the relevant central and regional ethics committees. The trial was conducted in agreement with the International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice (GCP).

PATIENT CONSENT STATEMENT

All patients provided written informed consent to participate in the study prior to screening.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

APPENDIX 1

Central and regional ethics committees

The following committees were responsible for granting ethics approval:

Country	Central Ethics Committee	Local Ethics Committee
Belgium	Comité d'éthique Érasme-ULB	UZ Gent Ethisch Comite
		Commissie Medische Ethiek UZ KU Leuven
Canada	N/A	Conjoint Health Research Ethics Board (CHREB), University of Calgary
		Research Ethics – Bannatyne, University of Mantoba
		Comité d'éthique de la recherche du CHUM
Germany	Geschäftsstelle der Ethikkommission der Friedrich-Alexander Universität Erlangen-Nürnberg	Ethik-Kommission an der Medizinischen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn
		Ethik-Kommission des Fachbereichs Medizin Universitätsklinikum der Goethe-Universität
		Ethik-Kommission bei der Landesärztekammer Rheinland-Pfalz
		Ethikkommission der Medizinischen Fakultät Heidelberg
Greece	Hellenic Republic Ministry of Health National Ethics Committee	Hospital's Scientific Committee General Hospital of Athens "LAIKO"
		Hospital's Scientific Committee General Hospital of Athens "Hippocratio"
		Scientific Committee University General Hospital of Larissa
Israel	N/A	Hadassah University Medical Center Helsinki Committee
		Sheba Medical Center Helsinki Committee
		Shaare Zedek Medical Center Helsinki Committee
		Tel Aviv Sourasky Medical Center Helsinki Committee
		Rabin Medical Center Helsinki Committee
		Rambam Health Care Campus Helsinki Committee
Italy	Spettabile Comitato Etico Ospedale San Gerardo	Spettabile Comitato Etico CESC Azienda Ospedaliera di Padova
		Spettabile Comitato Etico IRCCS Ospedale Casa Sollievo della Sofferenza
		Spettabile Comitato Etico A.O.U. di Bologna
		Spettabile Comitato Etico delle Marche
Spain	Drugs Research Ethics Committee of the Hospital Clinic of Barcelona	N/A
UK	Yorkshire & The Humber - Leeds East Research Ethics Committee	N/A
USA	Western Institutional Review Board (WIRB)	Tulane Human Research Protection Office Institutional Review Boards
		Chesapeake Institutional Review Board
		Mayo Clinic Institutional Review Boards
		UC Davis Institutional Review Board
		University of Tennessee Health Science Center (UTHSC) Institutional Review Board
		Georgetown-Howard Universities Center for Clinical and Translational Science Institutional Review Board

APPENDIX 2

Prohibited medications leading to patient exclusion

The following medications were prohibited within 3 months of the first visit and during the double-blind treatment period:

a. OCA, budesonide and other systemic corticosteroids, colchicine, mycophenolate mofetil, azathioprine, sulfasalazine, leflunomide, cyclophosphamide, fenofibrates and other fibrates, valproate, isoniazid, and nitrofurantoin.

b. Any biological agent within 12 weeks or five half-lives prior to Visit 1, whichever was longer. In the case of rituximab, use within 168 days (24 weeks) of Visit 1 or no recovery (level < 20% of pre-rituximab levels or below lower level of normal, whichever was lower) of CD19-positive B lymphocytes if the last dose of rituximab was more than 24 weeks prior to Visit 1.

c. Patients taking the following medications which are OAT1 and OAT3 substrates were not allowed on the study because of the underlying conditions these medications are used to treat: methotrexate, probenecid, aminohippurate, cephradine, cidofovir, adefovir, oseltamivir, acyclovir, ganciclovir, benzylpenicillin, cefaclor, ceftizoxime, bumetanide, famotidine, conjugated equine oestrogens, *liothyronine, ouabain, caspofungin, liotrix, romidepsin, fluvastatin, paclitaxel, docetaxel, cobimetinib, selexipag, ambrisentan, grazoprevir, technetium tc 99 m mebrofenin, and parachlorophenol. Should these drugs have become necessary during the treatment period, the treating physician was to make all efforts to use alternative drugs. However, where it was not feasible or desirable to change to an alternative, the patient was to be monitored closely for occurrence of adverse events possibly related to drug accumulation as well as clinical effects of increased exposure, and consideration was to be given to reducing the dose.

*Only equine oestrogen derivatives were prohibited. Synthetic oestradiol was allowed.

In addition, the following medications were prohibited during the double-blind treatment period:

d. Systemically administered potent CYP3A4 inhibitors: itraconazole, lopinavir/ritonavir, telaprevir, clarithromycin, ritonavir, ketoconazole, indinavir, conivaptan, and voriconazole.

e. Sensitive CYP3A4 substrates which have a narrow therapeutic range: alfentanil, astemizole, cisapride, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, systemic sirolimus, systemic tacrolimus, and terfenadine. However, should fentanyl or alfentanil use have become necessary during the study, and where it was not feasible or desirable to change to an alternative, use of fentanyl or alfentanil was permitted provided the dose was carefully titrated.

f. UGT inhibitors and inducers: atazanavir, rifabutin, carbamazepine, phenytoin, oxcarbazepine, nevirapine, methsuximide, and phenobarbital.

g. Pruritus medication: rifampicin, naltrexone. Antihistamines were permitted, if the dose was stable for at least 8 weeks before Visit 1 and throughout the study.

Note: these are not to be regarded as exhaustive lists. The FDA and other agencies maintain current lists, which can be referred to via their websites. Furthermore, these lists are anticipated to evolve as new drugs come to market and more is learned about the pharmacology of GKT137831 and other medications. Therefore, they are to be regarded as a minimum set of excluded and pre-cautioned concomitant medications.

APPENDIX 3

Study outcomes

Primary efficacy endpoint:

- Percentage change from baseline to Week 24 in serum GGT.

Secondary efficacy endpoints:

- Absolute and percentage change in serum GGT from baseline to each assessment.
- Absolute change in ELF score from baseline to Weeks 12 and 24.
- Absolute and percentage change in serum ALP from baseline to each assessment.
- Absolute and percentage change in serum levels of hsCRP and fibrinogen, from baseline to each assessment.
- Absolute and percentage change in serum ALT, AST, and conjugated and total bilirubin, from baseline to each assessment.
- Absolute and percentage change in fibrosis (FIB)-4 and AST to platelet ratio index (APRI) scores, from baseline to each assessment.
- Absolute and percentage change in liver stiffness, as assessed by vibration-controlled transient elastography (FibroScan® or similar technology), from baseline to Week 24, in subjects with values at baseline and Week 24.
- Absolute and percentage change in serum levels of collagen fragments indicative of collagen formation and degradation, from baseline to Weeks 12 and 24.
- Absolute and percentage change in quality of life, fatigue and pruritus scores based on the PBC-40 and pruritus Visual Analogue Scale (VAS), from baseline to Weeks 12 and 24.

Tertiary efficacy endpoints:

- Absolute and percentage change in total bile acid levels from baseline to Weeks 12 and 24.
- Proportion of subjects achieving a 15, 20, 30 and 40% reduction in serum ALP from baseline to each assessment.

- Proportion of subjects who meet the definition of PBC responder criteria applying the Paris I, Toronto I, Toronto II, Toronto III, Toronto IV, Mayo II, and Barcelona disease prognostic risk criteria at Weeks 12 and 24.

Exploratory endpoints:

- Absolute and percentage change in serum C4 and FGF19 from baseline to Weeks 12 and 24.

- Absolute and percentage change in serum interleukin (IL)-6 and cytokeratin-18 (CK-18), from baseline to Weeks 12 and 24.
- Absolute and percentage change in serum IgM, IL-4, IL-12, IL-17A and interferon γ, from baseline to Weeks 12 and 24.
- Assessment of metabolomics signatures.
- Assessment of additional biomarkers of interest.