

Efficacy and safety of PXL770, a direct AMP kinase activator, for the treatment of non-alcoholic fatty liver disease (STAMP-NAFLD): a randomised, double-blind, placebo-controlled, phase 2a study



Kenneth Cusi, Naim Alkhouri, Stephen A Harrison, Pascale Fouqueray, David E Moller, Sophie Hallakou-Bozec, Sebastien Bolze, Jean-Marie Grouin, Sophie Jeannin Megnien, Julie Dubourg, Vlad Ratziu

Summary

Background AMP kinase (AMPK) is an energy sensor implicated in regulation of lipid metabolism, inflammation, and insulin sensitivity. We aimed to assess efficacy and safety of PXL770, a novel direct AMPK activator, in patients with non-alcoholic fatty liver disease (NAFLD).

Methods STAMP-NAFLD, a randomised, double-blind, placebo-controlled phase 2a study, was done across 15 US clinical sites. Patients aged 18–75 years with liver fat content of at least 10% at baseline when assessed by MRI-proton density fat fraction (MRI-PDFF) were eligible. Patients were randomly assigned (1:1:1), via an interactive web response system, to receive oral PXL770 250 mg once daily, 250 mg twice daily, or 500 mg once daily, or matched placebo. Patients were stratified according to type 2 diabetes status and study site. The primary endpoint was relative change in liver fat content from baseline compared with placebo at week 12, assessed by MRI-PDFF. The primary endpoint was analysed in an ANCOVA model with treatment and stratification criteria as factors and baseline liver fat content as a covariate in the modified intention-to-treat population, defined as all as-randomised patients who received at least one dose of study treatment. Safety was analysed in the safety population, defined as all as-treated patients receiving at least one dose of the study treatment. The trial has been completed and the final results are reported. The trial is registered with ClinicalTrials.gov, NCT03763877.

Findings Between March 29, 2019, and March 13, 2020, 387 patients were screened, of whom 120 were included in the modified intention-to-treat and safety analyses (30 in the 250 mg once daily group, 30 in the 250 mg twice daily group, 29 in the 500 mg once daily group, and 31 in the placebo group). The mean relative change from baseline in liver fat content at week 12 was -1.1% in the placebo group, -1.0% in the 250 mg once daily group (mean difference versus placebo 0.1% [95% CI -15.4 to 15.7], $p=0.99$), -14.3% in the 250 mg twice daily group (-13.1% [-28.1 to 1.8], $p=0.084$), and -14.7% in the 500 mg once daily group (-13.5% [-28.5 to 1.4], $p=0.076$). At least one treatment-emergent adverse event occurred in 23 (77%) of 30 patients in the 250 mg once daily group, 20 (67%) of 30 patients in the 250 mg twice daily group, 21 (72%) of 29 patients in the 500 mg once daily group, and 21 (68%) of 31 patients in the placebo group. The most common treatment-emergent adverse event was diarrhoea (five [17%] of patients in the 250 mg once daily group, seven [23%] in the 250 mg twice daily group, six [21%] in the 500 mg once daily group, and none in the placebo group). No life-threatening events or treatment-related deaths occurred.

Interpretation PXL770 treatment did not meet the primary outcome of liver fat improvement compared with placebo. Treatment was well tolerated. Given indications that metabolic features improved with PXL770 treatment, AMPK activation might be a promising pharmacological target for patients with type 2 diabetes and NAFLD, and could also be considered for further assessment in patients with non-alcoholic steatohepatitis.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined by hepatic steatosis in the absence of secondary causes of liver fat accumulation, such as alcohol consumption.^{1,2} It is the leading cause of chronic liver disease and frequently progresses to non-alcoholic steatohepatitis (NASH), characterised by the additional presence of inflammation, hepatocellular damage

(ballooning), and fibrosis.³ NAFLD and NASH are associated with a marked increase in the risk of cardiovascular disease, hepatocellular carcinoma, and liver failure leading to transplantation.^{1,3} Currently, there are no approved pharmacological treatments for NAFLD and NASH.⁴

There is a growing consensus that lipotoxicity has an important role in the pathophysiology of NASH.^{5,6} Lipid

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Division of Endocrinology, Diabetes and Metabolism, University of Florida, Gainesville, FL, USA (Prof K Cusi MD); Malcom Randall Veterans Administration Medical Center, Gainesville, FL, USA (Prof K Cusi); Arizona Liver Health, Tucson, AZ, USA (N Alkhouri MD); Pinnacle Clinical Research, San Antonio, TX, USA (Prof S A Harrison MD); Poxel, Lyon, France (P Fouqueray MD, D E Moller MD, S Hallakou-Bozec PhD, S Bolze PharmD, J Dubourg MD); Department of Statistics, Université de Rouen, Rouen, France (J-M Grouin PhD); Summit Clinical Research, San Antonio, TX, USA (S Jeannin Megnien MD); Institute for Cardiometabolism and Nutrition, Sorbonne Université, Hôpital Pitié Salpêtrière, Paris, France (Prof V Ratziu MD)

Correspondence to:
Julie Dubourg, Poxel, 69007 Lyon, France
julie.dubourg@me.com

Research in context

Evidence before this study

Non-alcoholic fatty liver disease (NAFLD) is the most common type of chronic liver disease; its more advanced form, non-alcoholic steatohepatitis (NASH), includes liver inflammation, fibrosis, and cell death. There are no approved therapies for NAFLD and NASH at present. PXL770 is a first-in-class investigational drug in development for metabolic disorders; it is a selective small-molecule direct allosteric AMPK activator. We searched PubMed for randomised controlled trials that were published in English between Jan 1, 1980, and Aug 29, 2021, with the search terms “AMP kinase”, “direct activator”, and “clinical trial”. We found no previous randomised controlled trials investigating the effects of a direct AMPK activator.

Added value of this study

Our study, STAMP-NAFLD, was a proof-of-concept, randomised, placebo-controlled, phase 2a trial designed to assess the effects of PXL770 in patients with NAFLD with and without type 2 diabetes. In this 12-week study, oral administration of PXL770 did not significantly reduce liver fat content compared with placebo. In the large subset (41–47% in each cohort) of patients with coexisting type 2 diabetes, substantially greater degrees of efficacy (liver fat and enzyme level reductions) were observed; treatment with PXL770 also

resulted in clinically meaning improvements in hyperglycaemia along with evidence of enhanced insulin sensitivity. PXL770 treatment was well tolerated; adverse events were generally mild and balanced between groups. To our knowledge, this is the first reported clinical trial in which a direct AMPK activator has been studied in humans.

Implications of all the available evidence

There is a continuing unmet need for new approaches to treat NAFLD and NASH. Our results support the potential therapeutic utility and safety of direct AMPK activation for patients with NAFLD or NASH and related metabolic disorders, including type 2 diabetes. The results also show translation of AMPK activator effects on liver fat, liver enzymes, and hyperglycaemia from animals to humans. Taken together with the breadth of knowledge implicating AMPK as a therapeutic target, our results suggest that this modality can also be considered and tested in other disease states. Additional studies with this and other AMPK activators will be required to determine long-term potential benefits and the safety profile of this approach. Such studies would include an assessment of efficacy—based on liver biopsy derived histology—in patients with biopsy-proven NASH as well as studies designed to evaluate the degree and durability of metabolic efficacy in patients with type 2 diabetes that is not well controlled with available therapies.

accumulation in the liver is driven by high concentrations of circulating free fatty acids derived from the diet and released from adipose tissue, as well as excessive hepatic de-novo lipogenesis² in the context of insulin resistance and metabolic dysfunction.

Importantly, type 2 diabetes is a frequent comorbid condition. The prevalence of NAFLD in patients with type 2 diabetes is estimated to be 60–70%, and NASH with coincident type 2 diabetes confers greater risk of progression to end-stage liver disease and mortality than NASH alone.^{5,7,8} In addition, higher degrees of insulin resistance are present in patients with both NASH and type 2 diabetes than in patients with either disease alone.⁵ Insulin resistance also directly drives progression of NAFLD to NASH through increases in adipose tissue lipolysis, stimulation of de-novo lipogenesis, and potentiation of inflammation.^{2,9} Notably, a classic insulin sensitiser, pioglitazone, has shown efficacy in NAFLD–NASH progression,¹⁰ and several medicines that are currently being studied for NASH are already approved for type 2 diabetes and obesity.^{3,4}

AMP-activated protein kinase (AMPK) is a well known cellular energy sensor that directly inhibits de-novo lipogenesis and augments catabolic pathways, including lipid use, when activated by energy depletion.¹¹ Thus, AMPK activation is proposed as an approach to reduce liver steatosis through the inhibition of lipid

biogenesis and promotion of fatty acid oxidation.¹² Furthermore, AMPK has established insulin sensitising, antihyperglycaemic, anti-inflammatory, and antifibrotic activity in preclinical models.^{11,13} Accordingly, accumulating preclinical evidence in rodents and non-human primates has shown that administration of AMPK activators improves multiple parameters related to NAFLD and NASH.¹³ Downregulation of endogenous AMPK activity has also been reported in the context of metabolic dysfunction,^{14,15} suggesting the potential for a lower AMPK tone in patients with combined liver steatosis and type 2 diabetes. These elements support the concept that AMPK could be an ideal target for the treatment of NASH.

PXL770 is a novel small molecule being developed for the treatment of NASH and related metabolic disorders. It functions as a specific direct allosteric activator of human AMPK by binding directly to the heterotrimeric enzyme complex. In immune and hepatic stellate cells, PXL770 suppresses cytokine release and activation, respectively; in several animal models, oral administration of PXL770 has improved insulin sensitivity, hyperglycaemia, and several histopathological features of NASH.¹⁶ Studies in healthy volunteers have shown that PXL770 is safe and well tolerated with a pharmacokinetic profile that supports oral dosing (Bolze S, unpublished).

PXL770 is the first direct AMPK activator to be pursued as an investigational therapy for human diseases,

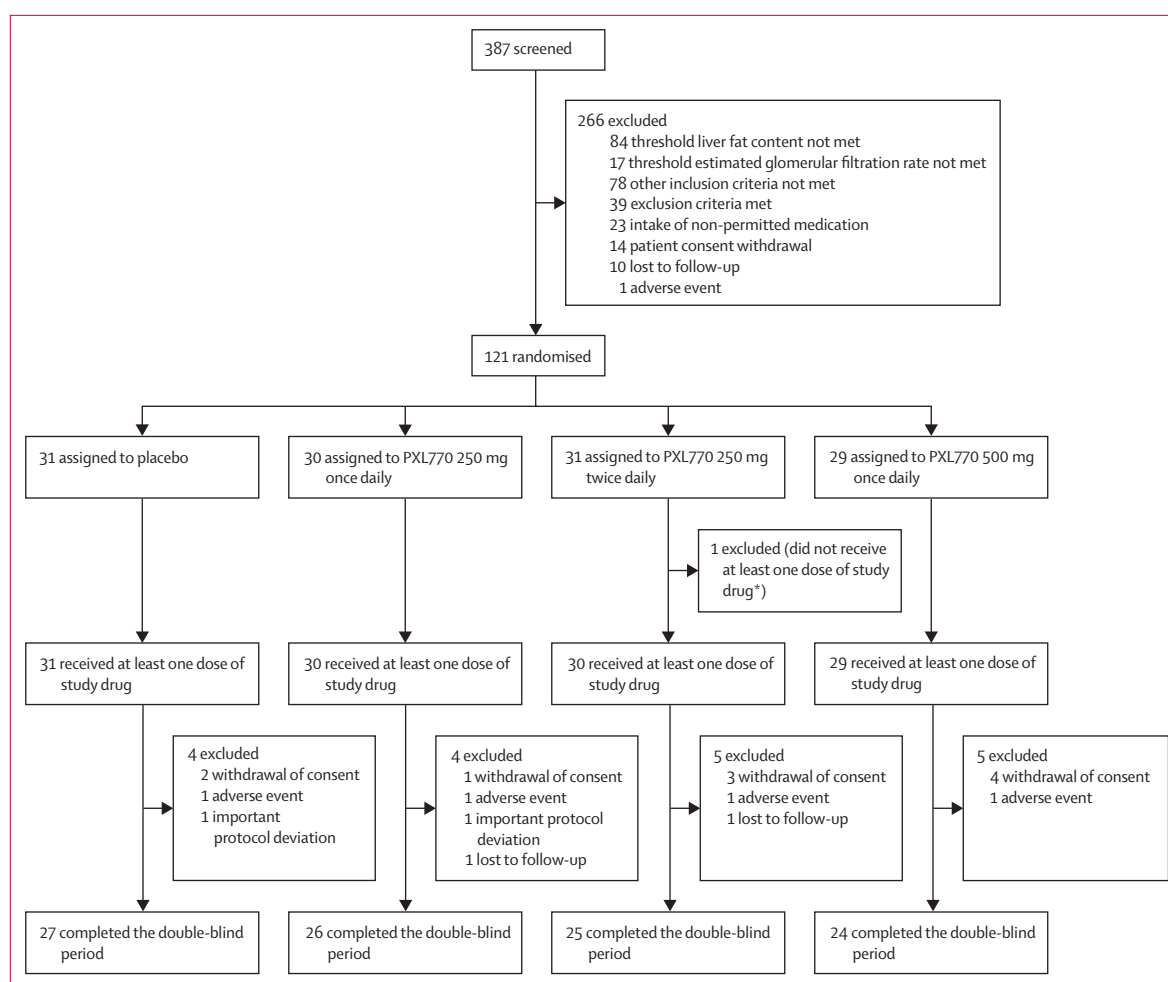


Figure 1: Trial profile

*Due to the occurrence of an adverse event before study drug administration.

including NAFLD and NASH and related conditions.^{11,17} We aimed to determine the effects of PXL770 on liver fat content versus placebo in patients with NAFLD. MRI-proton density fat fraction (PDFF) was used to measure effects on hepatic steatosis. To our knowledge, PXL770 is the first direct AMPK activator that has progressed to a clinical efficacy study so far.

Methods

Study design and patients

STAMP-NAFLD was a randomised, double-blind, placebo-controlled, phase 2a study of 12 weeks duration done across 15 clinical sites in the USA. Adults (aged 18–75 years) with liver fat content of 10% or higher assessed by MRI-PDFF, body-mass index of 25–50 kg/m², estimated glomerular filtration rate of at least 60 mL/min per 1.73 m², and elevated alanine aminotransferase (ALT) concentrations (>20 U/L in women and >30 U/L in men) were enrolled. The ALT eligibility criterion was secondarily removed following a

protocol amendment on Nov 20, 2019, when it was deemed to be too restrictive.

Patients were excluded if they had a relevant history of alcohol consumption (≥ 21 units of alcohol per week in males and ≥ 14 units per week in females for 2 years before screening, where a unit of alcohol is equal to 10 g pure alcohol); clinically significant acute or chronic liver disease unrelated to NASH (based on medical history, investigator expertise, and hepatitis serological screening); a history or presence of compensated or decompensated cirrhosis; a liver transplant; any cardiovascular event or evidence of active cardiovascular disease within 6 months of screening; history of more than 5% weight loss or bariatric surgery; and uncontrolled type 2 diabetes (glycated haemoglobin [HbA_{1c}] concentration of >9% [>75 mmol/mol]). Patients with type 2 diabetes were required to remain on a stable regimen of their previous antihyperglycaemic therapies during the study period. Non-permitted medications during the study included pioglitazone, insulin, GLP-1 receptor agonists, SGLT2 inhibitors, anticoagulant agents,

	Placebo (n=31)	PXL770 250 mg once daily (n=30)	PXL770 250 mg twice daily (n=30)	PXL770 500 mg once daily (n=29)
Age, years	51.5 (12.2)	52.5 (12.9)	50.6 (12.3)	55.7 (12.6)
Sex				
Male	15 (48%)	13 (43%)	7 (23%)	7 (24%)
Female	16 (52%)	17 (57%)	23 (77%)	22 (76%)
Race				
Asian	1 (3%)	1 (3%)	1 (3%)	0
Black or African American	1 (3%)	2 (7%)	3 (10%)	4 (14%)
White	27 (87%)	27 (90%)	25 (83%)	24 (83%)
Other	2 (6%)	0	1 (3%)	1 (3%)
Ethnicity				
Hispanic or Latino	7 (23%)	15 (50%)	13 (43%)	7 (24%)
Not Hispanic or Latino	24 (77%)	15 (50%)	17 (57%)	22 (76%)
Weight, kg	101.2 (19.8)	96.1 (18.4)	98.0 (19.7)	98.3 (18.2)
Body-mass index, kg/m ²	35.4 (6.0)	35.2 (6.3)	36.6 (6.3)	35.1 (4.9)
Hypertension	21 (68%)	19 (63%)	12 (40%)	20 (69%)
Dyslipidaemia	19 (61%)	14 (47%)	17 (57%)	20 (69%)
Type 2 diabetes	14 (45%)	13 (43%)	14 (47%)	12 (41%)
Duration of diabetes, years	2.2 (1.4–5.7)	6.7 (4.3–13.5)	4.0 (1.8–9.2)	3.5 (1.8–7.9)
HbA _{1c} , %; mmol/mol				
All patients	6.0% (1.1); 42 (12)	6.3% (1.1); 45 (12)	6.2% (0.8); 44 (9)	6.1% (0.8); 43 (9)
Patients with type 2 diabetes	6.6% (1.4); 49 (15)	7.1% (1.1); 54 (12)	6.8% (0.8); 51 (9)	6.7% (0.7); 50 (8)
Fasting plasma glucose, mmol/L				
All patients	6.5 (1.9)	6.7 (1.9)	6.5 (1.5)	6.3 (1.1)
Patients with type 2 diabetes	7.9 (1.9)	8.0 (2.2)	7.4 (1.7)	6.8 (1.3)
Liver fat content, %	21.8% (8.4)	16.0% (4.6)	20.3% (7.6)	18.7% (6.5)
Alanine aminotransferase, U/L	37.2 (19.1)	37.2 (22.1)	37.8 (36.5)	41.3 (34.7)
Elevated alanine aminotransferase*	12 (39%)	9 (30%)	7 (23%)	10 (34%)
Aspartate aminotransferase, U/L	24.1 (9.0)	25.3 (12.4)	27.2 (21.1)	28.0 (20.8)
Elevated aspartate aminotransferase*	5 (16%)	4 (13%)	4 (13%)	5 (17%)

Data are mean (SD), n (%), or median (IQR). HbA_{1c}=glycated haemoglobin. *The upper limit of the reference range was 41 IU/L for alanine aminotransferase and 34 IU/L for aspartate aminotransferase.

Table 1: Baseline characteristics of the modified intention-to-treat population

amiodarone, bile salt chelators, methotrexate, 400 units or more per day of vitamin E, corticosteroids with significant systemic effects (eg, oral or intravenous administration), any other medications known to affect liver function and steatosis at the investigator's discretion, strong inhibitors of CYP2C8 and CYP2C9, moderate inducers of CYP2C8 and CYP2C9, and any herbal drug or Chinese traditional medicine.

The study protocol was approved by one central ethics committee (Sterling Institutional Review Board, Atlanta, GA, USA) and three local ethics committees (Western Institutional Review Board in Puyallup, WA, USA, Duke University Health System Institutional in Durham, NC, USA; and AdventHealth Orlando Institutional Review Board in Maitland, FL, USA) before study initiation. The study was done in compliance with International Conference on Harmonisation E6 Good Clinical Practice. Written informed consent was obtained from all patients before beginning any study-related activities.

Randomisation and masking

Eligible patients were randomly assigned (1:1:1:1) to receive oral PXL770 either 250 mg once daily, 250 mg twice daily, or 500 mg once daily, or matched placebo. Patients were allocated to treatment groups via an interactive web response system (ClinTrak Interactive Response Technology, a MedSpace [Cincinnati, OH, USA] in-house system) and stratified by type 2 diabetes status (yes or no) and study site. The randomisation schedule was generated by an independent statistician before the first participant was screened. The randomisation schedule was generated using SAS PROC PLAN (version 9.4) through ClinTrak Interactive Response Technology. At randomisation, local investigators at clinical sites verified patient details and eligibility criteria, and entered the information into the interactive web response system to obtain a unique randomisation number. Study treatment was allocated according to the randomisation number. Allocation concealment was achieved by the use of capsules identical in appearance. Each patient received two capsules twice

daily to maintain strict blinding. Investigators, staff, patients, and the funder remained blinded throughout the study period.

Procedures

Dose selection was based on the exposure–efficacy relationship established during the preclinical pharmacological programme¹⁶ and the pharmacokinetics, safety, and tolerability observed in healthy participants during the phase 1 studies (Bolze S, unpublished). In this context, 250 mg once daily was shown to achieve an exposure within the lower part of the expected active dose range and 500 mg once daily at the higher end of the active dose range. The once daily regimen was expected to be the appropriate regimen based on a relatively long half-life of the compound (20–24 h). However, it was not fully determined whether the effects of PXL770 are mainly driven by the maximum plasma concentration or by the area under the curve because the half-life is shorter in rodents and most preclinical studies were done with a twice daily regimen. Therefore, it was deemed relevant to also investigate the activity of the top dose (500 mg per day) administered as a twice daily regimen.

After a 2-week screening period, all patients received oral placebo during a 4-week run-in period. After the run-in period, study treatment was administered for 12 weeks. Patients underwent MRI-PDFF testing before randomisation and at the end of treatment to assess liver fat content. Scans were performed by use of qualified and standardised instruments at high field strength (3.0 T or 1.5 T) without oral or intravenous contrast. Local MRI facilities completed a qualification and quality assurance process before performing study MRI examinations. All acquisition images were transferred to Medpace Core Laboratories (Cincinnati, OH, USA) for central calculation and measurement of MRI-PDFF using the method of Zhong and colleagues¹⁸ to estimate water and fat components. A 3 cm² region of interest was placed in each Couinaud segment. Central reading was blinded to treatment group, clinical data, study site of origin, and timepoint. All biochemical measurements were undertaken centrally at Medpace Reference Laboratories (Cincinnati, OH, USA). The upper limit of the reference range was 41 IU/L for ALT and 34 IU/L for aspartate aminotransferase (AST).

Patients were assessed (physical examination, vital signs, 12-lead electrocardiogram, and safety and efficacy laboratory assessments) at weeks 2, 4, and 8, and at the end of treatment on week 12, and at follow-up 1 week after the last dose.

Clinical sites accessed the electronic data capture system (designed by Medpace) to enter data. Specifically designed computer-generated checks (as well as manual edit checks) were used to identify data entry errors or other data inconsistencies. Data were reviewed on an ongoing basis. When flagged, necessary requests for

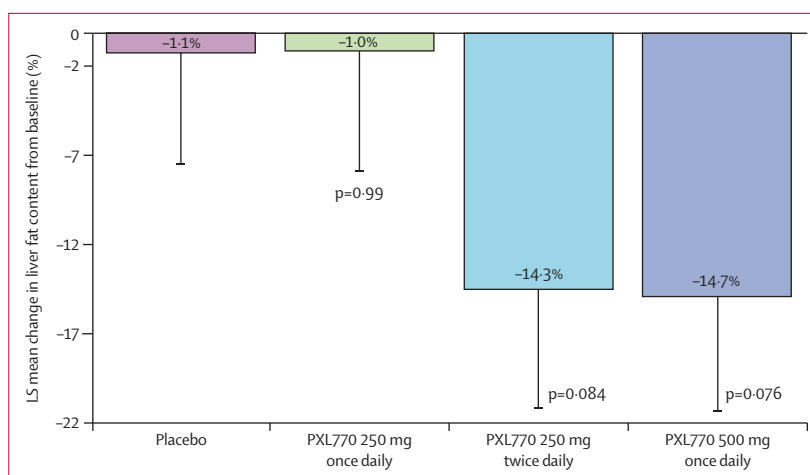


Figure 2: Relative change in liver fat content, assessed by MRI-PDFF, from baseline to week 12 in the modified intention-to-treat population

Error bars indicate SE values. p values represent changes versus placebo. LS=least squares. PDFF=proton density fat fraction.

clarifications or corrections were sent to the investigator at each clinical site via data queries within the electronic data capture system. After all queries were resolved and appropriate corrections or clarifications were made to the database, the database was considered to be clean. After all patients had either discontinued or completed their week 13 visits, the database was locked, and week 12 endpoints were read out.

In response to the COVID-19 pandemic, alternative processes to mitigate the impact of COVID-19 on the conduct of the study and to eliminate additional risks to patients were implemented by the funder on March 25, 2020. The main alternative processes were the possibility of virtual visits (phone calls) combined with laboratory assessments done at a local laboratory if in-person visits were not feasible for weeks 2, 4, 8, and 13 (except for the randomisation visit and the primary endpoint collection visit at week 12); the possibility to ship study medication, diaries, and other study material to patients; and the extension of the double-blind treatment period to a maximum duration of 20 weeks in total (ie, an additional 8 weeks).

Outcomes

The primary outcome was the relative change in the percentage of liver fat content from baseline to week 12 compared with placebo, as measured by MRI-PDFF. Secondary outcomes were absolute change from baseline in liver fat content, proportion of patients who achieved a clinically meaningful reduction of at least 30% in relative liver fat content, change from baseline in liver enzymes (AST and ALT), glycaemic parameters (fasting plasma glucose [FPG], HbA_{1c}, quantitative insulin sensitivity check index [QUICKI], and homeostatic model assessment for insulin resistance [HOMA-IR]), Fibrosis-4 index (FIB-4), and lipid parameters (total

	Placebo (n=31)	PXL770 250 mg once daily (n=30)	PXL770 250 mg twice daily (n=30)	PXL770 500 mg once daily (n=29)
Relative change in liver fat content, %				
Patients	31	30	30	29
Baseline, mean (SD)	21.8% (8.4)	16.0% (4.6)	20.3% (7.6)	18.7% (6.5)
Change from baseline, LS mean (SE)	-1.1% (6.3)	-1.0% (6.8)	-14.3% (6.7)	-14.7% (6.5)
Difference vs placebo, LS mean (SE)	..	0.1% (7.9)	-13.1% (7.6)	-13.5% (7.6)
95% CI	..	-15.4 to 15.7	-28.1 to 1.8	-28.5 to 1.4
p value vs placebo	..	0.99	0.084	0.076
Relative change in liver fat content*, %				
Patients	27	26	23	24
Baseline, median (IQR)	19.9% (12.2)	15.4% (7.2)	18.3% (4.9)	16.7% (13.1)
Change from baseline, median (IQR)	0.5% (24.1)	1.4% (27.2)	-8.3% (36.2)	-15.3% (42.9)
Difference vs placebo, location shift (95% CI)	..	-4.2% (-15.6 to 8.7)	-13.6% (-26.2 to 1.9)	-18.7% (-32.0 to -1.6)
p value vs placebo	..	0.55	0.10	0.039
MRI-PDFF				
Relative liver fat content reduction ≥30%, n (%)	2 (6%)	4 (13%)	4 (13%)	8 (28%)
Difference vs placebo, odds ratio (95% CI)	..	1.74 (0.27 to 11.27)	2.29 (0.37 to 14.21)	5.67 (1.00 to 32.15)
p value	..	0.56	0.37	0.050
Absolute change in liver fat content, %				
Patients	31	30	30	29
Baseline, mean (SD)	21.8% (8.4)	16.0% (4.6)	20.3% (7.6)	18.7% (6.5)
Change from baseline, LS mean (SE)	0.0% (1.1)	-0.2% (1.2)	-2.5% (1.2)	-2.4% (1.4)
Difference vs placebo, LS mean (SE)	..	-0.2% (1.4)	-2.6% (1.4)	-2.4% (1.4)
95% CI	..	-3.0 to 2.5	-5.3 to 0.1	-5.1 to 0.3
p value vs placebo	..	0.86	0.061	0.077
Alanine aminotransferase, IU/L				
Patients	27	26	23	24
Baseline, mean (SD)	36.0 (18.3)	38.2 (22.1)	37.9 (41.1)	42.9 (36.7)
Change from baseline, least-squares mean (SE)	1.0 (2.9)	0.0 (3.0)	0.3 (3.1)	-6.3 (3.0)
Difference vs placebo, LS mean (SE)	..	-0.9 (3.7)	-0.6 (3.8)	-7.3 (3.8)
95% CI	..	-8.3 to 6.4	-8.2 to 7.0	-14.9 to 0.3
p value vs placebo	..	0.80	0.87	0.058
Aspartate aminotransferase, IU/L				
Patients	27	26	23	24
Baseline, mean (SD)	23.3 (9.1)	25.7 (12.7)	27.3 (22.3)	28.7 (22.1)
Change from baseline, LS mean (SE)	-2.8 (2.0)	-1.7 (2.0)	-2.2 (2.1)	-7.0 (2.0)
Difference vs placebo, LS mean (SE)	..	1.1 (2.4)	0.6 (2.5)	-4.3 (2.5)
95% CI	..	-3.8 to 5.9	-4.4 to 5.5	-9.2 to 0.7
p value vs placebo	..	0.67	0.82	0.092

(Table 2 continues on next page)

cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides). These parameters were also analysed in the subgroup of patients with type 2 diabetes.

Safety endpoints included vital signs, physical examination, 12-lead electrocardiogram, clinical laboratory parameters, and adverse events. Adverse events were collected over the 12-week duration period and up to 1 week

after the last dose of study drug. Adverse events were coded by a coding specialist and reviewed by a medical monitor before the study was unblinded. Adverse events as well as medical and surgical history were coded to system organ class and preferred term using the Medical Dictionary for Regulatory Activities (version 23.0, COVID-19 update). Concomitant medications were coded to Anatomical

	Placebo (n=31)	PXL770 250 mg once daily (n=30)	PXL770 250 mg twice daily (n=30)	PXL770 500 mg once daily (n=29)
(Continued from previous page)				
FIB-4				
Patients	25	23	23	22
Baseline, mean (SD)	0.92 (0.45)	0.98 (0.54)	0.96 (0.31)	1.08 (0.48)
Change from baseline, LS mean (SE)	-0.11 (0.06)	-0.07 (0.06)	-0.09 (0.06)	-0.15 (0.06)
Difference vs placebo, LS mean (SE)	..	0.04 (0.07)	0.02 (0.07)	-0.04 (0.08)
95% CI	..	-0.11 to 0.19	-0.12 to 0.17	-0.19 to 0.11
p value vs placebo	..	0.57	0.76	0.57
Fasting plasma glucose, mmol/L				
Patients	27	26	23	24
Baseline, mean (SD)	6.4 (2.0)	6.6 (2.1)	6.6 (1.6)	6.2 (1.2)
Change from baseline, LS mean (SE)	0.36 (0.20)	0.19 (0.21)	-0.17 (0.21)	-0.16 (0.21)
Difference vs placebo, LS mean (SE)	..	-0.17 (0.26)	-0.53 (0.26)	-0.52 (0.26)
95% CI	..	-0.68 to 0.34	-1.05 to -0.02	-1.04 to 0.00
p value vs placebo	..	0.51	0.043	0.049
HbA_{1c}, %; mmol/mol				
Patients	28	26	23	24
Baseline, mean (SD)	6.0% (1.2); 42 (13)	6.3% (1.2); 45 (13)	6.2% (0.8); 44 (9)	6.1% (0.8); 43 (9)
Change from baseline, LS mean (SE)	0.05% (0.09); -0.5 (1.0)	-0.08 (0.10); -0.1 (1.1)	-0.18% (0.10); 2.0 (1.1)	-0.24% (0.10); -2.6 (1.1)
Difference vs placebo, LS mean (SE)	..	-0.13% (0.11); -1.4 (1.2)	-0.23% (0.12); -2.5 (1.3)	-0.29% (0.11); -3.2 (1.2)
95% CI	..	-0.35 to 0.09; -3.8 to 1.0	-0.46 to 0.00; -5.0 to 0.0	-0.51 to -0.06; -5.6 to -0.7
p value vs placebo	..	0.24	0.050	0.012
QUICKI				
Patients	27	23	23	23
Baseline, mean (SD)	0.298 (0.028)	0.297 (0.023)	0.294 (0.024)	0.298 (0.032)
Change from baseline, LS mean (SE)	-0.010 (0.003)	-0.006 (0.004)	-0.001 (0.004)	0.000 (0.003)
Difference vs placebo, LS mean (SE)	..	0.004 (0.004)	0.010 (0.004)	0.010 (0.004)
95% CI	..	-0.004 to 0.012	0.001 to 0.018	0.002 to 0.018
p value vs placebo	..	0.34	0.021	0.017
HOMA-IR				
Patients	27	23	23	23
Baseline, mean (SD)	7.96 (6.92)	6.99 (3.80)	7.82 (5.27)	8.64 (8.69)
Change from baseline, LS mean (SE)	0.64 (0.81)	-0.27 (0.87)	-0.53 (0.87)	-0.59 (0.86)
Difference vs placebo, LS mean (SE)	..	-0.92 (1.02)	-1.18 (1.02)	-1.23 (1.02)
95% CI	..	-2.94 to 1.11	-3.20 to 0.85	-3.26 to 0.80
p value vs placebo	..	0.37	0.25	0.23

(Table 2 continues on next page)

Therapeutic Chemical class and preferred term using the WHO Drug Dictionary (version Sep2018G B3).

Statistical analysis

Sample size calculation was based on the primary endpoint (ie, the relative change in the percentage of liver fat content from baseline to week 12). The primary objective was to show the superiority of at least one PXL770 dose over placebo with regard to the primary endpoint. The treatment difference assumption was based on previous published data.¹⁹ A sample size of 30 patients per randomised treatment group was required to achieve 90% power to show a significant treatment difference in the mean relative change in liver

fat content between PXL770 and placebo at the two-sided α level of 0.05, assuming a true difference of 30%, a SD of 30%, and a dropout rate of 20%. Given the trial phase, no adjustment for multiple comparisons between PXL770 doses and placebo was considered.

We analysed the relative change in liver fat content from baseline to week 12 in an ANCOVA model with treatment and stratification criteria as factors, and baseline liver fat content as a covariate in the modified intention-to-treat (ITT) population, defined as all as-randomised patients who received at least one dose of study treatment. We estimated missing MRI-PDFF data at week 12 using a multiple imputation procedure. Because there was only one post-randomisation value for the primary endpoint,

	Placebo (n=31)	PXL770 250 mg once daily (n=30)	PXL770 250 mg twice daily (n=30)	PXL770 500 mg once daily (n=29)
(Continued from previous page)				
Total cholesterol, mmol/L				
Patients	27	25	23	24
Baseline, mean (SD)	5.2 (1.3)	4.9 (1.1)	5.0 (0.9)	4.7 (1.1)
Change from baseline, LS mean (SE)	0.11 (0.17)	-0.14 (0.17)	0.06 (0.18)	0.08 (0.18)
Difference vs placebo, LS mean (SE)	..	-0.25 (0.22)	-0.05 (0.22)	-0.03 (0.22)
95% CI	..	-0.68 to 0.18	-0.49 to 0.39	-0.47 to 0.41
p value vs placebo	..	0.25	0.82	0.91
HDL cholesterol, mmol/L				
Patients	27	25	23	24
Baseline, mean (SD)	1.3 (0.3)	1.1 (0.2)	1.1 (0.3)	1.1 (0.3)
Change from baseline, LS mean (SE)	-0.01 (0.04)	-0.03 (0.04)	-0.09 (0.04)	0.00 (0.04)
Difference vs placebo, LS mean (SE)	..	-0.02 (0.05)	-0.08 (0.05)	-0.01 (0.05)
95% CI	..	-0.11 to 0.08	-0.17 to 0.02	-0.09 to 0.10
p value vs placebo	..	0.70	0.10	0.86
LDL cholesterol, mmol/L				
Patients	24	25	23	23
Baseline, mean (SD)	2.8 (1.0)	3.0 (1.0)	3.0 (0.9)	2.6 (1.0)
Change from baseline, LS mean (SE)	0.01 (0.14)	-0.09 (0.14)	0.16 (0.14)	0.09 (0.15)
Difference vs placebo, LS mean (SE)	..	-0.11 (0.18)	0.14 (0.18)	0.08 (0.18)
95% CI	..	-0.46 to 0.25	-0.21 to 0.50	-0.28 to 0.44
p value vs placebo	..	0.56	0.43	0.66
Triglycerides, mmol/L				
Patients	27	25	23	24
Baseline, mean (SD)	2.1 (0.9)	1.9 (0.8)	1.9 (0.8)	2.1 (1.0)
Change from baseline, LS mean (SE)	0.16 (0.22)	-0.01 (0.23)	0.02 (0.23)	-0.05 (0.23)
Difference vs placebo, LS mean (SE)	..	-0.16 (0.29)	-0.14 (0.29)	-0.20 (0.29)
95% CI	..	-0.73 to 0.41	-0.71 to 0.43	-0.77 to 0.37
p value vs placebo	..	0.57	0.63	0.49
FIB-4=Fibrosis-4 index. HbA _{1c} =glycated haemoglobin. HOMA-IR=homeostatic model assessment for insulin resistance. LS=least squares. PDFF=proton density fat fraction. QUICKI=quantitative insulin sensitivity check index. *Sensitivity analysis based on a non-parametric approach.				
Table 2: Change from baseline versus placebo in MRI-PDFF, liver enzymes, FIB-4, lipid parameters, and glycaemic parameters in the modified intention-to-treat population				

the missing pattern was monotone. The imputation model included treatment, stratification factors, and baseline liver fat content, and we used the fully conditional specification method. Missing data were imputed 100 times to generate 100 imputed datasets. The seed was 7704. We then analysed imputed datasets separately using the same model for the primary endpoint analysis (ANCOVA). We combined estimates across imputed datasets (using SAS PROC MIANALYZE) using Rubin's rule. Least-squares (LS) means with SEs, differences in LS means with SEs, 95% CIs, and the corresponding p values were calculated. Secondary endpoints were analysed in the modified ITT population. We did a sensitivity semi-parametric analysis in the modified ITT population, and the Hodges-Lehmann estimate along with its 95% CI, and the Wilcoxon test p values for two independent groups were provided for each pairwise comparison.

We did a responder analysis (response defined as a

≥30% relative reduction in liver fat content from baseline), in which response was analysed in a logistic regression model with treatment, stratification criteria as factors, and baseline liver fat content as a covariate, in the modified ITT population. Pairwise differences versus placebo were estimated as odds ratios along with their 95% CIs and p values. We used the same multiple imputation procedure as for the primary endpoint analysis to impute missing MRI-PDFF data at week 12 and then derive the patient status (responder vs non-responder).

Safety and tolerability analyses were done in the safety population (defined as all as-treated patients receiving at least one dose of the study treatment). All safety endpoints were analysed descriptively.

SAS (version 9.4) was used for all analyses. The trial is registered with ClinicalTrials.gov, NCT03763877.

Role of the funding source

The study was designed by the funder with the support of expert consultants in the NASH field. The funder had a role in data collection, data interpretation, data analysis, and writing of the report.

Results

387 patients were screened between March 29, 2019, and March 13, 2020. Of the 121 individuals considered eligible, 30 were randomly assigned to receive 250 mg PXL770 once daily, 31 to receive 250 mg PXL770 twice daily, 29 to receive 500 mg PXL770 once daily, and 31 to receive placebo (figure 1). One (3%) of 31 patients in the 250 mg PXL770 twice daily group did not receive at least one dose of double-blind study treatment; thus, 120 patients were included in the modified ITT analysis and in the safety analysis. Overall, 102 (84%) of 121 patients completed the double-blind treatment period and 19 (16%) patients withdrew during the double-blind treatment period (figure 1). The double-blind treatment period was extended up to 20 weeks for 18 patients. The numbers of patients included by clinical site are shown in the appendix (p 4).

The majority of patients were female (78 [65%]), white (103 [86%]), and not Hispanic or Latino (78 [65%]). The mean age was 52.5 years (SD 12.5; table 1). In the subgroup of patients with type 2 diabetes, the mean baseline values for liver fat content, ALT, and AST were similar to the mean values for the overall study population (appendix p 1). Baseline HbA_{1c} and FPG were consistent with well controlled diabetes (table 1). The median treatment duration was 85.0 days (IQR 83.5–88.0).

In the modified ITT population, the placebo-subtracted LS mean relative change from baseline in liver fat content at week 12 (the primary endpoint) was 0.1% (95% CI –15.4 to 15.7; $p=0.99$) in the 250 mg once daily group, –13.1% (–28.1 to 1.8; $p=0.084$) in the 250 mg twice daily group, and –13.5% (–28.5 to 1.4; $p=0.076$) in the 500 mg once daily group (figure 2; table 2). Significant LS mean relative changes in liver fat content from baseline were observed at the doses of 250 mg twice daily ($p=0.033$) and 500 mg once daily ($p=0.023$). In the sensitivity analysis based on a non-parametric approach, the Hodges-Lehmann median difference in liver fat content at week 12 compared with placebo was –4.2% ($p=0.55$) in the 250 mg once daily group, –13.6% ($p=0.10$) in the 250 mg twice daily group, and –18.7% ($p=0.039$) in the 500 mg once daily group (table 2).

In the modified ITT population, the placebo-subtracted LS mean absolute change from baseline in liver fat content at week 12 was –0.2% (95% CI –3.0 to 2.5; $p=0.86$) in the 250 mg once daily group, –2.6% (–5.3 to 0.1; $p=0.061$) in the 250 mg twice daily group, and –2.4% (–5.1 to 0.3; $p=0.077$) in the 500 mg once daily group (table 2).

The number of patients who achieved a 30% or more relative reduction of liver fat content was two (6%) of

See Online for appendix

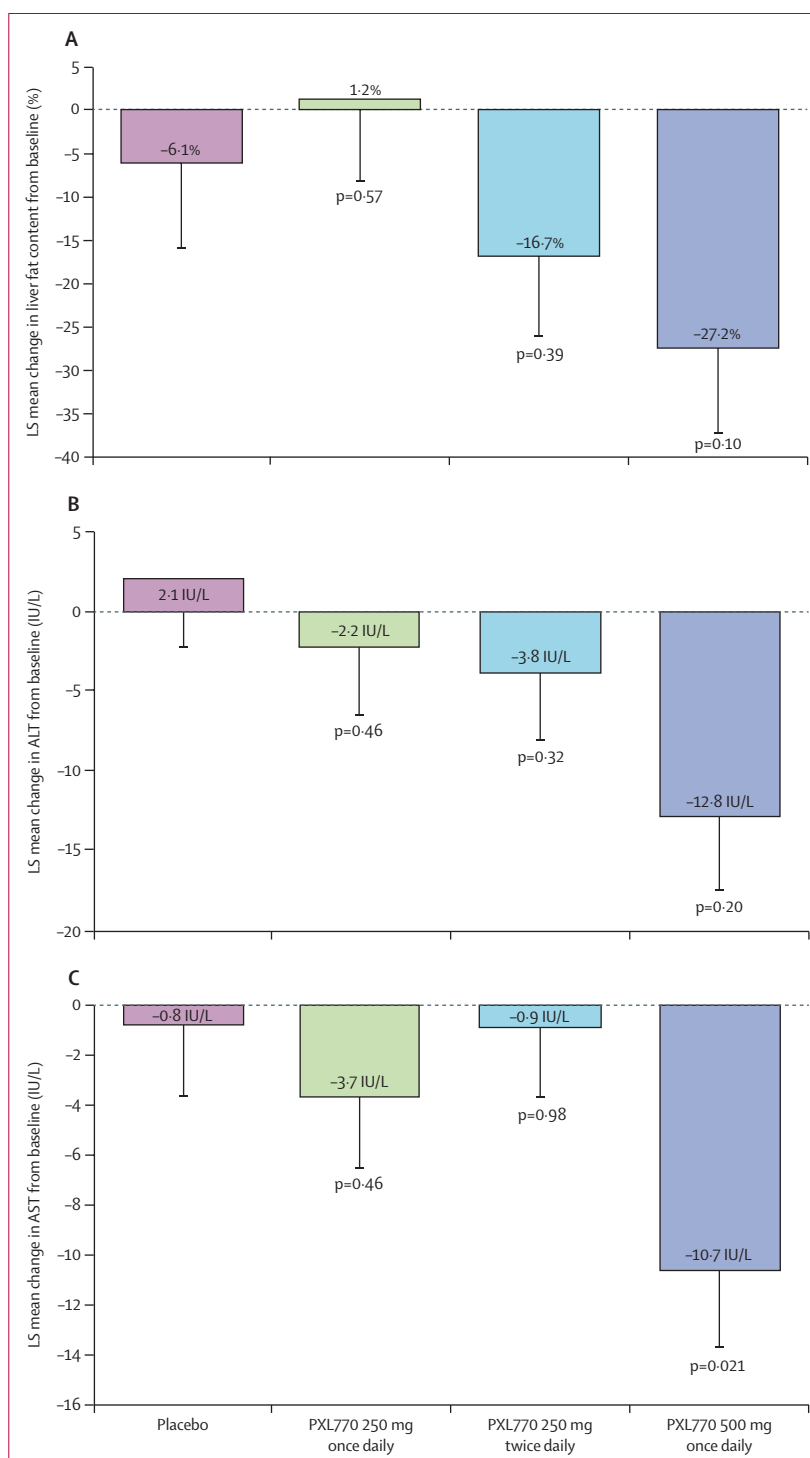


Figure 3: Relative change in liver fat content, assessed by MRI-PDFF, and liver enzymes from baseline to week 12 in the subpopulation of patients with type 2 diabetes
Change in liver fat content (A), ALT (B), and AST (C). Error bars indicate SE values. p values represent changes versus placebo. ALT=alanine aminotransferase. AST=aspartate aminotransferase. LS=least squares. PDFF=proton density fat fraction.

	Placebo (n=31)	PXL770 250 mg once daily (n=30)	PXL770 250 mg twice daily (n=30)	PXL770 500 mg once daily (n=29)
Patients with treatment-emergent adverse events	21 (68%)	23 (77%)	20 (67%)	21 (72%)
Mild	14 (45%)	13 (43%)	10 (33%)	12 (41%)
Moderate	7 (23%)	10 (33%)	9 (30%)	7 (24%)
Severe	0	0	1 (3%)	2 (7%)
Patients with serious adverse events	0	0	2 (7%)	1 (3%)
Patients with related serious adverse events	0	0	1 (3%)	0
Treatment-emergent adverse events occurring in >10% of patients				
Diarrhoea	0	5 (17%)	7 (23%)	6 (21%)
Nausea	1 (3%)	2 (7%)	1 (3%)	4 (14%)
Headache	0	3 (10%)	4 (13%)	2 (7%)
Migraine	0	0	3 (10%)	0
Patients with ALT increase*	0	1 (3%)	2 (7%)	1 (3%)

Data are n (%). ALT=alanine aminotransferase. *ALT increase is defined as an increase of greater than or equal to three times the upper limit of normal for patients with normal baseline value or greater than or equal to three times baseline for patients with elevated ALT at baseline.

Table 3: Adverse events in the safety population

31 patients in the placebo group, four (13%) of 30 patients in the 250 mg once daily group, four (13%) of 30 patients in the 250 mg twice daily group, and eight (28%) of 29 patients in the 500 mg once daily group (table 2).

In patients treated with PXL770, the mean numerical changes from baseline in ALT ranged from 0.0 IU/L (SE 3.0) to -6.3 IU/L (3.0) versus a mean 1.0 IU/L (2.9) increase with placebo. No apparent trends in AST concentrations or FIB-4 values were noted (table 2).

Dose-responsive changes in both placebo-subtracted FPG and HbA_{1c} changes from baseline were evident and were significant for FPG with both the 250 mg twice daily and 500 mg once daily groups and for HbA_{1c} with the 500 mg once daily group (table 2). There were no significant changes in HOMA-IR; however, significant improvements in QUICKI were observed with both the 250 mg twice daily and 500 mg once daily groups (table 2).

There were no changes in bodyweight versus baseline in any of the treatment groups; in addition, no meaningful changes in standard measures of lipids, including total triglycerides, total cholesterol, LDL cholesterol, or HDL cholesterol, were observed (table 2).

We next analysed the effects of PXL770 on both NAFLD-related and glycaemic parameters in the subpopulation of patients with type 2 diabetes (figure 3; appendix pp 2–3) as part of a preplanned secondary analysis. The placebo-subtracted LS mean relative change from baseline in liver fat content at week 12 was 7.3% (95% CI -18.0 to 32.5; $p=0.57$) in the 250 mg once daily group, -10.6% (-34.6 to 13.4; $p=0.39$) in the 250 mg twice daily group, and -21.1% (-46.5 to 4.2; $p=0.10$) in the 500 mg once daily group (appendix pp 2–3). A greater proportion of patients achieved a relative reduction of liver fat

content of 30% or more in the 500 mg once daily group than in the placebo group (appendix pp 2–3). Dose-responsive decreases in ALT, AST, and FIB-4 were observed in patients with type 2 diabetes who received PXL770; these effects were significant versus placebo for both ALT and AST in the 500 mg once daily group (appendix pp 2–3).

At week 12, treatment with 500 mg once daily was associated with significant improvements in several glycaemic parameters versus placebo (appendix pp 2–3). Despite nearly normal mean baseline FPG (7.6 mmol/L) and HbA_{1c} (6.8%; 51 mmol/mol), treatment with PXL770 was associated with apparent dose-responsive decreases in both FPG and HbA_{1c} in patients with coexisting type 2 diabetes. These effects were significant versus placebo in the 500 mg once daily group (appendix pp 2–3). Treatment with 500 mg once daily was also associated with a significant improvement in QUICKI, with a similar effect, albeit not significant, when the HOMA-IR index was used (appendix pp 2–3).

No life-threatening events or deaths occurred during the study. At least one treatment-emergent adverse event occurred in 23 (77%) of 30 patients in the 250 mg once daily group, 20 (67%) of 30 patients in the 250 mg twice daily group, 21 (72%) of 29 patients in the 500 mg once daily group, and 21 (68%) of 31 patients in the placebo group (table 3). The most frequently reported treatment-emergent adverse events in the PXL770 treatment groups were diarrhoea (five [17%] of patients in the 250 mg once daily group, seven [23%] in the 250 mg twice daily group, six [21%] in the 500 mg once daily group, and none in the placebo group), nausea, headache, and ALT increase (table 3).

Two patients in the 250 mg twice daily group had a serious adverse event: one event of sepsis considered severe and unrelated to the study treatment, and one ALT increase considered mild and related to the study treatment. One participant in the 500 mg once daily group had a serious adverse event: atrial fibrillation with sinus node dysfunction considered severe and unrelated to the study treatment.

An increase in the incidence of diarrhoea, which was of mild intensity, was observed in all groups treated with PXL770 without an apparent dose effect. A slight increase in the incidence of headache was also observed but only one event in the 250 mg once daily group was considered to be related to the study drug.

Transient and isolated increases in ALT concentration (three times greater than the upper limit of normal or three times greater than baseline) were observed in four patients treated with PXL770: three patients with a normal baseline ALT concentration and one patient with elevated ALT baseline concentration (table 3). In all of these cases, ALT increases were observed at weeks 4–8 and were accompanied by a smaller increase in AST concentrations. There were no changes in other liver enzymes; no changes in bilirubin, alkaline phosphatase concentration, or prothrombin time were noted. These cases were all

asymptomatic. In two patients, ALT returned to (or below) the individual's baseline while treatment was still ongoing after approximately 1 month. In two cases, investigators decided to discontinue treatment and ALT returned to baseline within the same timeframe (1 month). The individual data for these ALT increases are shown in the appendix (p 5).

Asymptomatic hypoglycaemia events, from self-monitoring of blood glucose, were reported in three patients (one in the 250 mg once daily group, one in the 250 mg twice daily group, and one in the 500 mg once daily group). No clinically relevant events in other safety laboratory assessments, physical examination, or electrocardiograms were noted.

Discussion

The STAMP-NAFLD study addressed the specific hypothesis that direct activation of AMPK would lead to improvements in hepatic steatosis and potentially affect other parameters related to hepatic inflammation and liver cell injury, such as aminotransferase concentrations. In this exploratory, proof of concept 12-week trial, oral treatment with PXL770 led to modest decreases in liver fat content, as measured by MRI-PDFF, with an apparent pattern of dose-response between the three tested doses. However, the results of the primary analysis were not significant. In a preplanned non-parametric sensitivity analysis, the placebo-subtracted relative reduction in liver fat content from baseline was significant in the 500 mg once daily dose group but not in the other groups. Reductions in liver enzymes at the 500 mg once daily dose were not significant.

Given that direct AMPK activators, including PXL770, enhance insulin sensitivity and ameliorate hyperglycaemia in models of type 2 diabetes,¹¹ we did subgroup analyses specifically in patients with type 2 diabetes. These patients accounted for 41–47% of patients in each group. We observed numerically greater effects with a potentially clinically relevant least-squares mean relative reduction in liver fat content of -27.2% (SE 9.6) from baseline along with a high proportion (58%) of patients achieving a 30% or more decrease in liver fat content at 500 mg once daily. Significant (versus placebo) decreases in ALT (-14.9 IU/L) and AST (-9.9 IU/L) were also seen at this dose. Although dose-responsive decreases were observed across dose groups, only the dose of 500 mg once daily group was significant. Additionally, in this subpopulation of patients with type 2 diabetes, significant placebo-subtracted decreases were observed in both FPG and HbA_{1c} along with evidence of improvement in insulin sensitivity.

Potentially enhanced effects of PXL770 to improve liver fat content and lower liver enzyme concentrations in patients with type 2 diabetes seem to be consistent with the published literature documenting that overnutrition, insulin resistance, and systemic inflammation are associated with lower levels of AMPK activity in key

tissues, including adipose, skeletal muscle, and liver;^{14,15} moreover, hyperglycaemia alone is sufficient to suppress AMPK activity.²⁰ Thus, greater responses to direct AMPK activation might be expected to occur in patients with diabetes than in those with lesser degrees of metabolic dysfunction.

The effects of PXL770 in patients with coexisting NAFLD and type 2 diabetes seem similar to those of other investigational oral drugs in trials in which populations of patients with NAFLD were studied for similar periods of time. For example, 12-week data reported with resmetirom, a thyroid hormone receptor- β (THR β) agonist, revealed a -33% change in liver fat content (-23% placebo-subtracted) and an -8.2 IU/L (-3.0 placebo-subtracted) mean decrease in ALT.²¹ In a 12-week study of MET-409, a farnesoid X receptor (FXR) agonist, greater liver fat content reductions (up to -55%) were observed; however mean ALT concentration were increased (28%) with a 50 mg dose versus decreased (-25%) at 80 mg.²² Importantly, the degree of HbA_{1c} lowering (up to -0.64% placebo-subtracted) achieved in this trial with PXL770 is likely to be clinically meaningful, given that patients already had well controlled type 2 diabetes at baseline. With approved antihyperglycaemic therapies for type 2 diabetes, decreases in HbA_{1c} are less pronounced when baseline glycaemia is only modestly abnormal; for example, in phase 2 and 3 studies, sitagliptin, an approved DPP-4 inhibitor lowered HbA_{1c} by approximately -0.5% when baseline values were less than 8.0% versus 0.7 – 0.9% when baseline HbA_{1c} was greater than 8.5% .²³ The apparent insulin-sensitising effects of PXL770 are also potentially relevant because insulin resistance is key driver of NAFLD and NASH.⁹ Given the increasing burden of NAFLD and NASH associated with type 2 diabetes, which is currently estimated to affect 6.4 million people in the USA,⁸ medicines that offer the potential to target both conditions in parallel would be an important addition to the future range of treatment options.

AMPK has been linked to numerous pleiotropic effects on key cellular pathways, such as lipid and glucose metabolism, inflammation, fibrogenesis, and autophagy.¹¹ Thus, AMPK activation has been proposed as a therapeutic strategy for a range of metabolic disorders, neurodegenerative diseases, kidney diseases, and certain cancers, including hepatocellular carcinoma.¹¹ Because of complex pathogenic relations with metabolic dysfunction and subclinical inflammation, NAFLD is a unique condition for exploring the potential therapeutic benefit of AMPK activation.^{11,13} Clinical effects observed with PXL770 were consistent with the mechanism of action of this molecule. AMPK activation is expected to reduce steatosis through the regulation of de-novo lipogenesis and the induction of lipid oxidation. Preclinical studies in rodents that tested PXL770 and related molecules showed reduced liver fat content and improvements in other

NASH-related parameters.^{13,16} Although the amount of liver fat is not predictive of disease progression during the natural course of the disease, the reduction in liver fat with a pharmacological intervention has been associated with higher odds of NASH resolution,²⁴ although this outcome might be dependent on the precise mechanism of action.²⁵ Chronic hepatic inflammation and hepatocyte injury are the main mechanisms that drive fibrosis progression. In this regard, it is noteworthy that AMPK activators such as PXL770 exert anti-inflammatory and antifibrotic effects in preclinical studies, such as direct suppression of inflammation and activation in human macrophages and hepatic stellate cells, respectively.¹⁶ With regard to glucose lowering and insulin sensitisation, a wealth of preclinical observations indicate that AMPK activation promotes insulin action and glucose uptake in skeletal muscle,¹¹ including a potential direct link between AMPK and insulin signalling.²⁶ Moreover, direct AMPK activator compounds, including PXL770, have consistently shown an effect to lower glucose in animal models of type 2 diabetes.^{11,16}

Notably, the commonly used type 2 diabetes medicine, metformin, has also been shown to activate AMPK in preclinical model systems.²⁷ Metformin has been studied in several small-scale clinical trials with only modest reductions in ALT and no clear effects on steatosis (or other features of NASH in trials in which histopathology was assessed).²⁸ Importantly, recent literature indicates that key in-vivo effects of metformin might not require AMPK and that other discrete mechanisms mediating its antihyperglycaemic efficacy are implicated.^{29,30} Moreover, metformin is an indirect activator of AMPK, unlike PXL770, which directly interacts with, and activates, the heterotrimeric enzyme complex.¹⁶ Therefore, we contend that a lack of effect of metformin to ameliorate NASH in previous trials should not preclude the further assessment of PXL770 in biopsy-proven NASH.

PXL770 was well tolerated with only a modest increase in mild gastrointestinal adverse events. A transient increase in ALT concentration was observed in four patients. These events were not associated with clinical symptoms or with increases in total bilirubin or alkaline phosphatase. In addition, there was no apparent relationship with dose, with adverse events occurring in all treatment groups. Similar events of isolated aminotransferase increases have been reported in other clinical trials in NASH, including with FXR agonists²² and a THR β agonist.³¹ These findings also displayed a pattern similar to those in the current study—increases occurring within the first 4–8 weeks after randomisation and typically in association with substantial decreases in liver fat. These findings have led us to speculate that such effects might represent an adaptive response to rapid hepatic de-lipidation.

STAMP-NAFLD was designed as a proof of concept, exploratory, non-invasive trial, and therefore the inherent limitations were the relatively small sample size, the short 12-week treatment duration, and the absence of adjustment

for multiple group comparisons. Patients included in this study were also not well representative of populations (eg, Hispanic, Latin-American, and South Asian) that are known to be more substantially affected by NAFLD.^{32,33} In addition, the study was not designed to address potential differences in sex or reproductive status and enrolled a higher than expected proportion of females. Given important differences in the severity of NAFLD and NASH relating to these parameters,³⁴ future trials will need to carefully encompass this concept. Other limitations included no further measurements of cardiovascular disease risk (subclinical atherosclerosis) and the absence of more robust predictors of fibrosis such as liver stiffness.³⁵ Of note, with the current (capsule) formulation used in this study, a food effect was observed and reduced the exposure by about 30% (Bolze S, unpublished). The highest dose level of 500 mg once daily was therefore not likely to have exceeded the range of exposures associated with optimal efficacy in animals; thus, it is possible that greater efficacy could have been achieved by using alternative formulation avoiding the food effect or increasing exposures. The trial was intended to provide preliminary confirmation of clinical and biological benefits of AMPK activation in the whole population of patients with NAFLD and in the subgroup of patients who also had type 2 diabetes. The results are therefore exploratory and hypothesis generating and need to be confirmed in larger, subsequent trials. Such trials should include formal assessments of liver histopathology with rigorous methodology, larger sample size, and longer treatment duration. In addition, we believe plasma exposures can be increased, as noted previously. Importantly, AMPK activation could complement other mechanisms of action, such as FXR, THR β , and glucagon-like peptide receptor agonists. For each of these classes of drugs, preclinical data suggest additive effects with PXL770 (Hallakou-Bozec S and Moller DE, unpublished).

The study had notable strengths, particularly with respect to providing the first evidence for clinical translation of the metabolic effects of a direct AMPK activator in humans. Rigorous methodology included the use of MRI-PDFF, an accurate and quantitative measurement of liver fat content, as an objective and reliable primary endpoint.

In conclusion, 12 weeks of oral treatment with PXL770 was well tolerated with an acceptable adverse event profile. In patients with NAFLD, reductions in liver fat content in those receiving higher doses of PXL770 were not significant compared with placebo. Patients receiving PXL770 500 mg once daily had significant improvements in fasting plasma glucose, HbA_{1c}, and QUICKI. The effects of PXL770 were apparently greater in patients with coexisting type 2 diabetes than in the whole cohort. These results support the further investigation of PXL770 as a possible treatment for type 2 diabetes or for NASH, in particular for the large number of patients with NASH and coexisting type 2 diabetes, in whom the severity and

progression of disease results in a high unmet medical need.

Contributors

KC was involved in conceptualisation and supervision of the study, and writing, review, and editing of the report. NA contributed to study investigation, and writing, review, and editing of the report. SAH and SJM were responsible for data interpretation, and writing, review, and editing of the report. PF contributed to the conceptualisation, methodology, and supervision of the study, project administration, and writing, review, and editing of the report. DEM was responsible for data interpretation, writing of the original draft of the report, and writing, review, and editing of the report. SH-B and SB contributed to conceptualisation of the study, and writing, review, and editing of the report. J-MG contributed to conceptualisation, methodology, and supervision of the study, data curation, and writing, review, and editing of the report. JD contributed to conceptualisation, methodology, and supervision of the study, data curation, project administration, writing of the original draft of the report, writing, review, and editing of the report. VR contributed to conceptualisation of the study, data interpretation, and writing, review, and editing of the report. The corresponding author, all co-authors, and the funder had full access to the all data in the study and accept responsibility to submit for publication. JD, PF, and J-MG fully accessed and verified the underlying data.

Declaration of interests

KC reports grant and research support from Cirius, Echosens, Inventiva, Novartis, Novo Nordisk, Poxel, and Zydus, and is a consulting adviser for Allergan, Altimmune, Arrowhead, AstraZeneca, Bristol Myers Squibb (BMS), Boehringer Ingelheim, Coherus, Eli Lilly, Fractyl, Hanmi, Genentech, Gilead, Intercept, Janssen, Pfizer, Poxel, Prosciento, Madrigal, and Novo Nordisk. NA reports grant and research support from 89Bio, Akero, Axcella, BI, BMS, Celgene, Gilead, Galmed, Galectin, Genfit, Enanta, Enyo, Hanmi, Inventiva, Madrigal, Merck, NGM Bio, Novo Nordisk, Pfizer, Poxel, and Zydus; is a consulting adviser for 89Bio, Fibronostics, Gilead, Intercept, Pfizer, Terns, and Zydus; and reports educational fees from AbbVie, Alexion, CLDF, Gilead, Intercept, and Simply Speaking. SAH reports grant and research support from Akero, Axcella, Cirius, Civi Biopharma, Cymabay, Enyo, Galectin, Galmed, Genfit, Gilead, Hepion, Hightide, Intercept, Madrigal, Metacrine, NGM, Northsea, Novartis, Novo Nordisk, Poxel, Sagimet, and Viking; is a consulting adviser for 89 Bio, AgomAB, Akero, Alentis, Alimientiv, Altimmune, Arrowhead, Axcella, Boston Pharmaceuticals, B Riley FBR, Canfit, Chronwell, Civi Biopharma, Corcept, Cymabay, Echosens, Enyo, Fibronostics, Foresite Labs, Fortress, Galectin, Galmed, Genfit, Gilead, GNS Healthcare, Hepion, Hightide, Histoindex, Indalo, Inipharma, Intercept, Ionis, Kowa Research Institute, Madrigal, Medpace, Metacrine, Microba, NGM, Northsea, Novartis, Novo Nordisk, Nutrasource, PathAI, Piper Sandler & Co, Poxel, Prometic, Ridgeline, Sagimet, Sonic Incytes Medical, Terns, Theratechnologies, and Viking; and holds shares in Akero, Chronwell, Cirius, Galectin, Genfit, Hepion, Histoindex, Metacrine, NGM, Northsea, PathAI, and Sonic Incytes Medical. PF, DEM, SH-B, and SB are employees of and shareholders in Poxel. J-MG is a consulting adviser for Poxel. JD is a former employee of and shareholder in Poxel. VR reports grant and research support from Gilead and is a consulting adviser for Boehringer Ingelheim, Galmed, Genfit, Poxel, Intercept, Novo-Nordisk, Terns, Alexion, Alnylam, Theratechnologies, NGMBio, Sagimet, Prosciento, Inventiva, Hanmi, Covance, and 89 Bio. SJM declares no competing interests.

Data sharing

Upon reasonable request and with the publication of this study, access to additional data from this study may be granted via contact with Pierre Theurey (pierre.theurey@poxelpharma.com). Data to be made available could potentially include de-identified participant data, data dictionary, or other specified data sets as well as study protocol, statistical analysis plan, informed consent form. Specific requests for data would require approval of a proposal and signed data access agreement.

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