Safety and efficacy of once-weekly efruxifermin versus placebo in non-alcoholic steatohepatitis (HARMONY): a multicentre, randomised, double-blind, placebo-controlled, phase 2b trial

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Summary

Background Fibroblast growth factor 21 (FGF21) regulates metabolism and protects cells against stress. Efruxifermin is a bivalent Fc–FGF21 analogue that replicates FGF21 agonism of fibroblast growth factor receptor 1c, 2c, or 3c. The aim of this phase 2b study was to assess its efficacy and safety in patients with non-alcoholic steatohepatitis (NASH) and moderate (F2) or severe (F3) fibrosis.

Methods HARMONY is a multicentre, randomised, double-blind, placebo-controlled, 96-week, phase 2b trial that was initiated at 41 clinics in the USA. Adults with biopsy-confirmed NASH, defined by a non-alcoholic fatty liver disease activity score (NAS) of 4 or higher and scores of 1 or higher in each of steatosis, ballooning, and lobular inflammation, with histological stage F2 or F3 fibrosis, were randomly assigned (1:1:1), via an interactive response system, to receive placebo or efruxifermin (28 mg or 50 mg), subcutaneously once weekly. Patients, investigators, pathologists, site staff, and the sponsor were masked to group assignments during the study. The primary endpoint was the proportion of patients with improvement in fibrosis of at least 1 stage and no worsening of NASH, based on analyses of baseline and week 24 biopsies (liver biopsy analysis set [LBAS]). A sensitivity analysis evaluated the endpoint in the full analysis set (FAS), for which patients with missing biopsies were considered non-responders. This trial is registered with ClinicalTrials.gov, NCT04767529, and is ongoing.

Findings Between March 22, 2021, and Feb 7, 2022, 747 patients were assessed for eligibility and 128 patients (mean age 54·7 years [SD 10·4]; 79 [62%] female and 49 male [38%]; 118 [92%] white; and 56 [41%] Hispanic or Latino) were enrolled and randomly assigned to receive placebo (n=43), efruxifermin 28 mg (n=42; two randomised patients were not dosed because of an administrative error), or efruxifermin 50 mg (n=43). In the LBAS (n=113), eight (20%) of 41 patients in the placebo group had an improvement in fibrosis of at least 1 stage and no worsening of NASH by week 24 versus 15 (39%) of 38 patients in the efruxifermin 28 mg group (risk ratio [RR] 2·3 [95% CI 1·1–4·8]; p=0·025) and 14 (41%) of 34 patients in the efruxifermin 50 mg group (2·2 [1·0–5·0]; p=0·036). Based on the FAS (n=128), eight (19%) of 43 patients in the placebo group met this endpoint versus 15 (36%) of 42 in the efruxifermin 28 mg group (RR 2·2 [95% CI 1·0–4·8]; p=0·033) and 14 (33%) of 43 in the efruxifermin 50 mg group (1·9 [0·8–4·3]; p=0·123). The most frequent efruxifermin-related adverse events were diarrhoea (16 [40%] of 40 patients in the efruxifermin 28 mg group and 17 [40%] of 43 patients in efruxifermin 50 mg group *vs* eight [19%] of 43 patients in the placebo group; all events except one were grade 1–2) and nausea (11 [28%] patients in the efruxifermin 28 mg group and 18 [42%] patients in the efruxifermin 50 mg group *vs* ten [23%] patients in the placebo group; all grade 1–2). Five patients (two in the 28 mg group and three in the 50 mg group) discontinued due to adverse events. Serious adverse events occurred in four patients in the 50 mg group; one was defined as drug related (ulcerative esophagitis in a participant with a history of gastro-oesophageal reflux disease). No deaths occurred.

Interpretation Efruxifermin improved liver fibrosis and resolved NASH over 24 weeks in patients with F2 or F3 fibrosis, with acceptable tolerability, supporting further assessment in phase 3 trials.

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Introduction

Non-alcoholic steatohepatitis (NASH) is a progressive form of non-alcoholic fatty liver disease (NAFLD), characterised by excessive accumulation of fat in hepatocytes.[1,2] NASH is associated with hepatocyte injury (ballooning), inflammation, and often fibrosis.[3] The mechanisms of pathogenesis and progression of NASH are complex, and ultimately converge within the liver. In

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See Online for appendix

Research in context

Evidence before this study

Liver fibrosis due to non-alcoholic steatohepatitis (NASH) is a leading cause of liver cirrhosis and end-stage liver disease globally. No therapy has been approved by the European Medicines Agency or the US Food and Drug Administration for treating NASH and associated liver fibrosis. Fibroblast growth factor 21 (FGF21) signalling can reverse many features of NASH pathogenesis, improving adipose tissue metabolism, restoring insulin sensitivity, reducing liver fat, and protecting hepatocytes from lipotoxicity-related stress. Unlike agents that include a single FGF21 moiety per molecule of analogue, efruxifermin is a bivalent analogue consisting of two covalently linked FGF21 chains, with higher affinity for its receptors and reduced dissociation.

Our phase 2a trial of efruxifermin, published in 2021, showed that efruxifermin significantly reduced hepatic fat in patients with fibrosis stage 1–3 NASH, with an acceptable safety profile. We searched MEDLINE, from Jan 1, 2017, to April 31, 2023, without any language restrictions, using the search terms "clinical trial", "FGF21 analog", "FGFR agonist", "NASH", "fatty liver", and "efficacy", for clinical trials evaluating the efficacy of FGF21 analogues or balanced agonists of the receptors FGFR1c, 2c, and 3c in patients with NASH. There were reports of non-invasive markers of reduced liver fat, injury, and fibrosis after treatment with an FGFR1c-specific agonist antibody (BFKB8488A), and with a pegylated FGF21 analogue, pegbelfermin, which did not appear to be a balanced agonist of FGF21's receptors due to concentration in the liver relative to periphery. The efficacy of aldafermin, an FGF19 analogue with activity at FGFR1c and FGFR4 (not a receptor of FGF21) had been evaluated in patients with biopsy-confirmed NASH.

Added value of this study

This phase 2b study showed that 24 weeks of efruxifermin treatment produced significant regression of fibrosis and resolution of steatohepatitis in patients with NASH.
The results from this study reproduced the results of hepatic fat normalisation and the reductions in markers of liver injury and fibrosis reported from our phase 2a studies.
The combination of improvements in liver and whole-body metabolic health, including enhanced insulin sensitivity, better glycaemic control, and improved lipid profile and modest weight loss, appears unique to NASH therapeutic agents in late-stage development.

Implications of all the available evidence

Effective treatment of liver fibrosis due to NASH is a major health-care need. In this 24-week phase 2b study with serial liver biopsies, efruxifermin showed significant histopathological improvements with broad metabolic improvements that could support sustained remission of NASH. Based on these data, efruxifermin will be evaluated in a phase 3 study of longer duration that assesses regression of fibrosis and resolution of steatohepatitis in patients with pre-cirrhotic or cirrhotic NASH.

hepatocytes, lipotoxicity and associated oxidative stress cause endoplasmic reticulum stress, activation of pro-apoptotic pathways, and release of inflammatory mediators, leading to differentiation of hepatic stellate cells into collagen-secreting myofibroblasts.[4] Continuously high rates of collagen deposition result in liver fibrosis, potentially leading to cirrhosis.[2,5] Later stages of NASH are associated with increased risk of hepatocellular carcinoma, end-stage liver-related events, major adverse cardiac events, and mortality.[6]

Fibroblast growth factor 21 (FGF21) is an endocrine member of the FGF15/19 subfamily of fibroblast growth factors.[2] Liver is the major source of circulating FGF21, although other tissues contribute, particularly when stressed metabolically.[7] FGF21 regulates glucose and lipid metabolism and whole-body energy homoeostasis, acting on tissues related to metabolic function.[8] During metabolic stress, FGF21 protects cells by increasing mitochondrial capacity, inducing antioxidant pathways, and restoring proteostasis.[9,10] By so doing, FGF21 prevents hepatocyte cell death, inflammation, and fibrosis, which are characteristic of NASH.

Efruxifermin is a 92 kDa human IgG1 Fc–FGF21 fusion protein with a pharmacokinetic half-life of approximately 3 days and sustained pharmacological effects, enabling once-weekly dosing.[2,11,12] In vitro, efruxifermin is a balanced agonist of FGF21's cognate receptors: FGFR1c, FGFR2c, and FGFR3c.[11] In a 16-week phase 2a trial (BALANCED) of patients with NASH and fibrosis stage (F1–F3),[13] administration of efruxifermin 28 mg, 50 mg, or 70 mg significantly reduced liver fat and improved whole-body lipid and glucose metabolism. In a study of 30 participants with compensated cirrhosis, efruxifermin 50 mg was associated with histopathological improvements after 16 weeks of treatment, including reversal of cirrhosis in four of 12 patients in the treated group (*vs* zero of five patients the placebo group).[14] In these studies, efruxifermin was associated mainly with mild or moderate nausea and diarrhoea.

Given the indications of improvement in histopathological features after only 16 weeks of efruxifermin administration, this phase 2b study (HARMONY) evaluated the effects of 24 weeks of efruxifermin 28 mg or efruxifermin 50 mg, versus placebo, on liver histology in participants with NASH and F2 or F3 fibrosis.

Methods

Study design and participants

HARMONY is a 96-week multicentre, randomised, double-blind, placebo-controlled, parallel-group, phase 2b

study, which was initiated at 41 clinics in the USA (appendix pp 3–4). Herein, we report the primary efficacy and safety analyses at week 24 of the ongoing 96-week study. Biopsies will be collected at week 96 from patients continuing in the study and the results will be reported in a future publication.

A central institutional review board (WCG IRB, 20203807) provided ethics approval of the study and all protocol amendments. All participants provided written informed consent before enrolment. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was consistent with the International Conference on Harmonization of Good Clinical Practice and applicable regulatory requirements.

Eligible adults (aged 18–75 years) had NASH, F2 or F3, an NAFLD activity score (NAS) of 4 or higher, and scores of at least 1 in each of steatosis (scored 0–3), ballooning (scored 0–2), and lobular inflammation (scored 0–3; confirmed by biopsies collected within 26 weeks of randomisation). Histopathology was scored on the basis of NASH-Clinical Research Network (CRN) staging and grading criteria.[15] Participants were also required to have a history or presence of two or more of the components of metabolic syndrome (ie, obesity, dyslipidaemia, high blood pressure, or high fasting glucose) or type 2 diabetes, as well as hepatic fat fraction (HFF) of 8% or higher by MRI proton density fat fraction (MRI-PDFF), controlled attenuation parameter (CAP) of at least 300 dB/m, and median liver stiffness of more than 8·5 kPa by vibration controlled transient elastography (VCTE; FibroScan, Echosens, Paris, Ille de France, France). Fibrosis stage 4 (F4), history of decompensated liver disease, liver transplantation, hepatocellular carcinoma, or other causes of liver disease, including autoimmune or viral hepatitis, were exclusionary. Full eligibility criteria are listed in the appendix (pp 5–7).

Randomisation and masking

Patient randomisation was performed by an interactive response technology (IRT) system (Endpoint Clinical, Wakefield, MA, USA). Randomisation was stratified by the presence or absence of type 2 diabetes and baseline fibrosis stage (F2 *vs* F3). Within each of the four strata, patients were randomly assigned (1:1:1) to receive efruxifermin 28 mg, efruxifermin 50 mg, or matching placebo using a block size of six. Site personnel obtained the patient's identification number and blinded study drug assignment from the IRT. Investigational product was dispensed by site personnel to patients in a blinded manner (ie, neither were aware of the contents of the dispensed product, and the appearance of all three possible treatment assignments was identical). Patients, investigators, pathologists, site staff, and the sponsor remained masked to group assignments during the course of the study.

For more on the **study** see https://classic.clinicaltrials.gov/ct2/show/NCT04767529?term=NCT04767529&draw=2&rank=1

Procedures

Efruxifermin 28 mg (Akero Therapeutics, South San Francisco, CA, USA), efruxifermin 50 mg, or

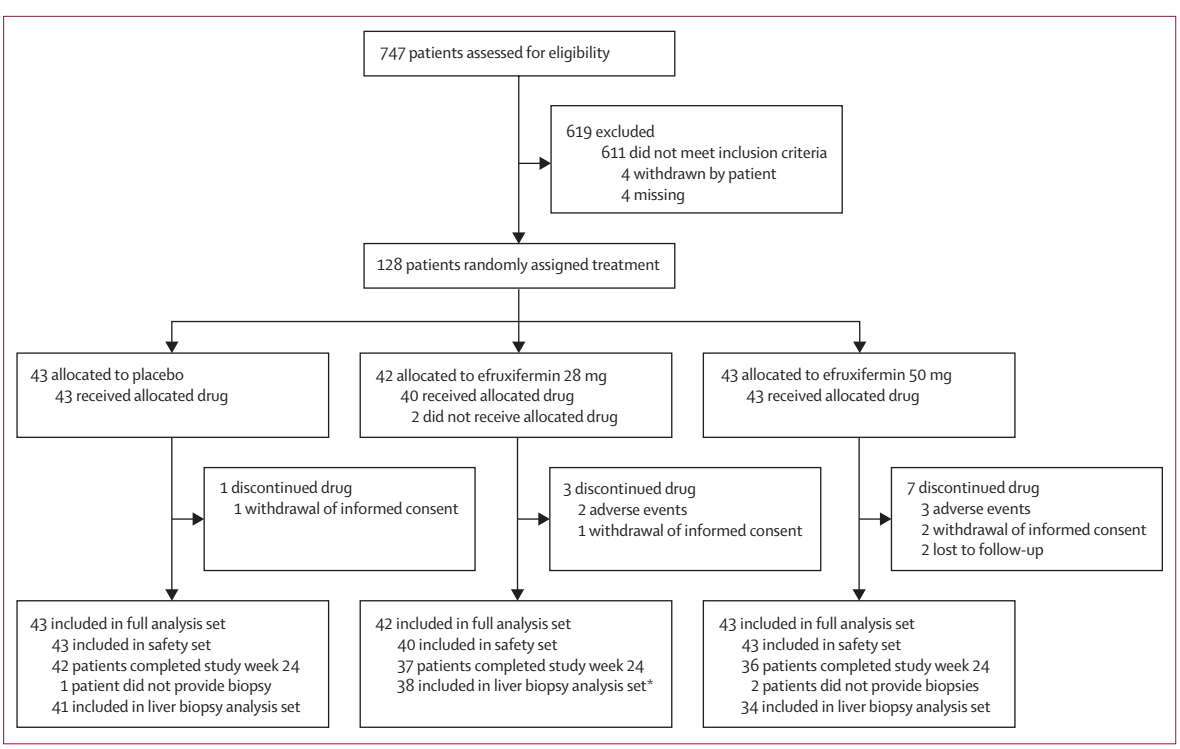


Figure 1: **Trial profile**
*One patient who did not complete week 24 did provide a post-baseline biopsy

	Placebo (n=43)	Efruxifermin 28 mg (n=42)	Efruxifermin 50 mg (n=43)	Total (n=128)
Age, years	55·0 (10·1)	56·5 (9·3)	52·4 (11·4)	54·7 (10·4)
Sex				
Female	27 (63%)	29 (69%)	23 (54%)	79 (62%)
Male	16 (37%)	13 (31%)	20 (47%)	49 (38%)
Ethnicity				
Hispanic or Latino	15 (35%)	17 (40%)	20 (47%)	52 (41%)
Non-Hispanic or Latino	28 (65%)	25 (60%)	23 (53%)	76 (59%)
Race				
White	39 (91%)	38 (91%)	41 (95%)	118 (92%)
Black or African American	2 (5%)	1 (2%)	1 (2%)	4 (3%)
Asian	2 (5%)	3 (7%)	0	5 (4%)
Native Hawaiian or Other Pacific Islander	0	0	0	0
American Indian or Alaskan Native	0	0	0	0
Other	0	0	1 (2%)	1 (1%)
Bodyweight, kg	107·6 (25·6)	103·9 (22·7)	102·8 (21·1)	104·8 (23·2)
BMI kg/m²	38·7 (7·7)	38·3 (6·9)	37·2 (6·6)	38·0 (7·0)
Type 2 diabetes	28 (65·1)	32 (76·2)	30 (69·8)	90 (70·3)
Glycated haemoglobin A_{1c}, %	6·8 (1·1)	6·8 (1·0)	6·7 (1·2)	6·8 (1·1)
Liver Histology				
Patients with F2	13 (30%)	15 (36%)	16 (37%)	44 (34%)
Patients with F3	30 (70%)	27 (64%)	27 (63%)	84 (66%)
NAFLD activity score	5·4 (1·2)	5·1 (1·0)	5·6 (1·1)	5·4 (1·1)
Non-invasive measures				
Enhanced liver fibrosis score	9·8 (0·7)	9·7 (0·8)	9·8 (0·8)	9·8 (0·8)
Median liver stiffness by vibration-controlled transient elastography (FibroScan), kPa	14·5 (6·2)	13·8 (5·2)	16·0 (7·1)	14·8 (6·2)
Hepatic fat fraction by MRI proton density fat fraction, %	17·1 (6·4)	18·5 (6·9)	17·5 (6·4)	17·7 (6·5)
Pro-C3, µg/L	16·5 (6·1)	15·3 (5·5)	18·4 (8·0)	16·7 (6·7)
Adiponectin, µg/mL	3·4 (2·0)	3·5 (1·6)	3·5 (1·5)	3·5 (1·7)
Alanine aminotransferase, U/L	62·2 (41·7)	49·7 (23·3)	63·3 (34·3)	58·5 (34·3)
Aspartate aminotransferase, U/L	57·0 (45·0)	41·8 (18·2)	52·4 (30·0)	50·5 (33·4)
Urate, mg/dL	5·6 (1·4)	5·7 (1·3)	5·7 (1·6)	5·6 (1·4)
Triglycerides, mg/dL	169·7 (87·3)	158·3 (48·8)	154·1 (68·7)	160·7 (70·0)
HDL cholesterol, mg/dL	42·2 (9·7)	41·8 (6·1)	40·5 (10·0)	41·5 (8·8)
LDL cholesterol, mg/dL	94·2 (33·8)	96·1 (29·5)	110·7 (35·5)	100·4 (33·7)
Medication use				
Statin use (yes)	21 (48·8)	22 (52·4)	14 (32·6)	57 (44·5)
Antidiabetic medication use (yes)	28 (65%)	30 (75%)	30 (70%)	88 (70%)
GLP-1 analogues	7 (16%)	5 (12%)	4 (9%)	16 (13%)
SGLT2 inhibitors	8 (19%)	6 (14%)	5 (12%)	19 (15%)

Data are n (%) or mean (SD).

Table 1: **Patient demographic and clinical characteristics at baseline (full analysis set)**

matching placebo was administered subcutaneously into the abdomen once weekly by health-care professionals either at the study clinic or via home health care. Liver biopsies were collected at screening (called the baseline biopsy, obtained within 6 months of day 1; day 1 was the baseline visit for most other measures) and at week 24. All liver biopsies were digitised and read to consensus by two independent pathologists, who were masked to patients and treatment groups (appendix p 18). Screening biopsies were read only once at the time of inclusion. To reduce temporal bias when reading week-24 biopsies, a proportion of screening biopsies were randomly shuffled in with week 24 biopsies.

Blood samples were collected at specified timepoints (baseline [day 1 visit]; weeks 1, 4, 8, 12, 16, 20, and 24; early termination; and day 30 follow-up for patients who terminated early) to assess markers of liver injury and function, markers of glucose and lipid metabolism, non-invasive markers of fibrosis, and safety parameters, such as chemistries (eg, sodium, potassium, blood urea nitrogen), haematology (eg, haematocrit, complete blood count), and coagulation panels. Imaging assessments, including liver fat content by MRI-PDFF and liver stiffness by VCTE, were performed at baseline and week 24.

Patients remained on existing medications, including anti-diabetic medicines, statins, GLP-1 receptor agonists, and SGLT2 inhibitors, which were required to be maintained at a stable dose for a minimum of 3 months before collection of biopsies to determine trial eligibility, and throughout randomisation.

Outcomes

The primary endpoint at week 24 was improvement in liver fibrosis by 1 or more stages without worsening of NASH, defined as no increase in score for any one of the components of NAS—namely, ballooning, inflammation, or steatosis.

Secondary endpoints (prespecified) assessed at week 24 were: proportion of patients with NASH resolution, defined as score of 0 for ballooning, 0 or 1 for inflammation, and any value for steatosis without worsening of liver fibrosis (as determined by the NASH-CRN criteria); proportion of patients who had improvement (by ≥1 stage in NASH-CRN fibrosis score) in liver fibrosis; change from baseline in HFF by MRI-PDFF; non-invasive markers of fibrosis (enhanced liver fibrosis [ELF] score, N-terminal type-III collagen pro-peptide [ProC3], NIS4 [a blood-based non-invasive test to determine risk of NASH and NAS ≥4 and F ≥2 among patients with metabolic risk factors], and liver stiffness by FibroScan); glycaemic control and insulin sensitivity (glycated haemoglobin [HbA_{1c}], C-peptide, adiponectin, and HOMA-IR); lipid metabolism (triglycerides, non-HDL-C, HDL-C, and LDL-C); change from baseline in bodyweight; and safety, tolerability, and immunogenicity of efruxifermin. Key exploratory (prespecified) endpoints were the proportion of patients with resolution of NASH and improvement in fibrosis, improvement in fibrosis by at least 2 stages without worsening of NASH, change from baseline in non-invasive markers of liver injury (alanine aminotransferase [ALT], aspartate aminotransferase [AST], gamma glutamyl transferase [GGT], alkaline phosphatase [ALP], bilirubin, and urate), and normalisation of HFF (ie, ≤5% HFF). Post-hoc analyses evaluated the proportion of patients with elevated serum levels of ALT (>30 U/L male, >19 U/L female) and AST (>30U/L, male and female)

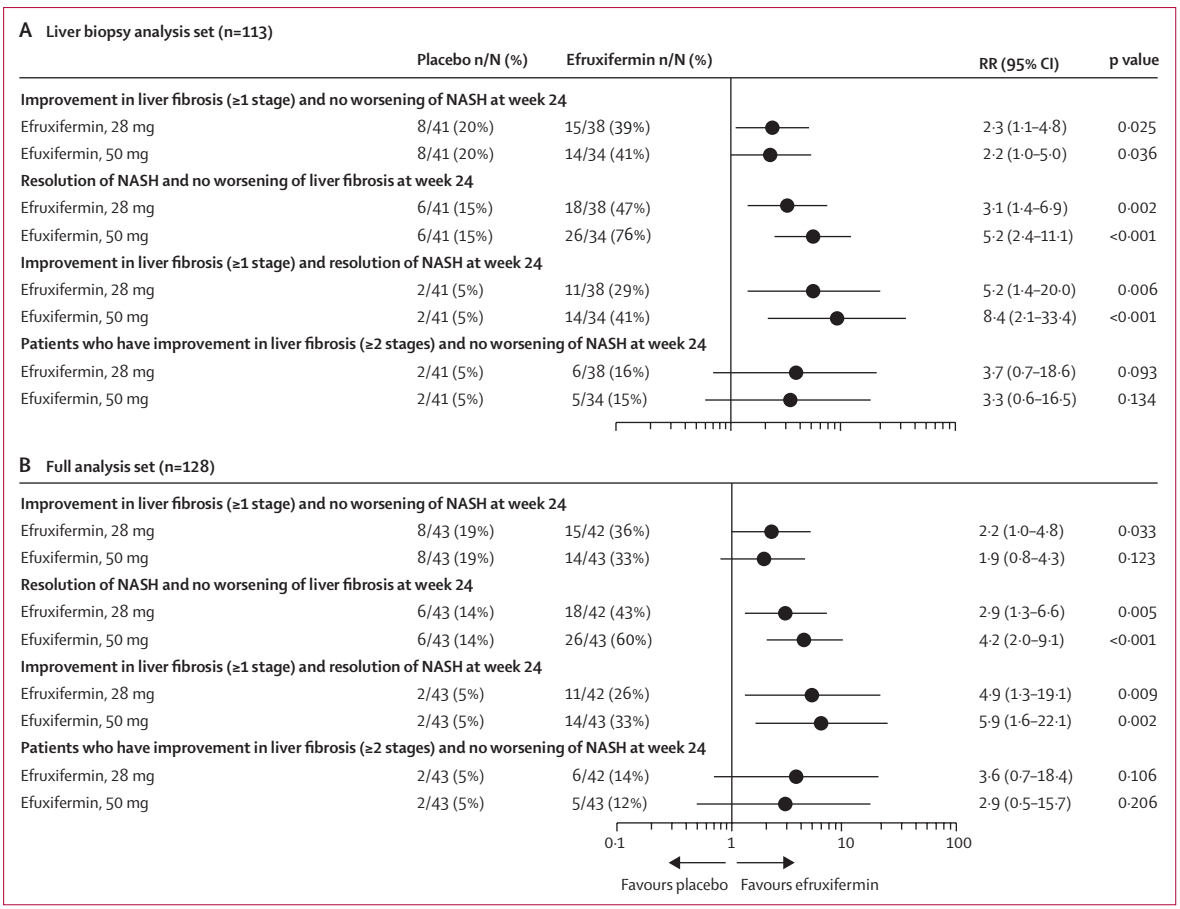


Figure 2: **Proportions of patients meeting histological endpoints in efruxifermin *vs* placebo at week 24**
Liver biopsy analysis set (n=113). (B) Full analysis set (n=128). NASH=non-alcoholic steatohepatitis. RR=risk ratio.

at baseline whose levels were normalised (ALT ≤30 U/L male, ≤19 female; AST ≤30 U/L, male and female) by efruxifermin; the association of ALT or AST normalisation with resolution of NASH and no worsening of fibrosis; subgroup analyses for proportion of patients who reached the primary endpoint by GLP-1 use at baseline; and changes in bone biomarkers over time (type I collagen C-telopeptide [CTX-1] and procollagen I N-terminal propeptide [P1NP]) in male patients, pre-menopausal female patients, and post-menopausal subgroups.

Safety and tolerability were evaluated on the basis of incidence of treatment-emergent adverse events; use of concomitant medications at every visit and throughout the study; clinical laboratory tests, including routine chemistry and haematology at every clinic visit; focused laboratory assessments, such as markers of bone metabolism (CTX-1 and P1NP at baseline and weeks 4, 12, and 24); vital signs (heart rate, systolic and diastolic blood pressure, bodyweight) at every clinic visit; triplicate blood pressure measurements at baseline and weeks 12 and 24; electrocardiographs (at baseline and week 24); and incidence of anti-efruxifermin antibodies ([ADAs] at baseline and weeks 8, 16, and 24).

Adverse events were graded based on version 5.0 of the Common Terminology Criteria for Adverse Events. Triplicate blood pressure assessments were gathered from the same arm, with at least 2 min rest between measurements.

Statistical analysis

It was estimated that approximately 60% of the patients in each efruxifermin dose group and 20% of those in the placebo group would reach the primary endpoint, based on results of the phase 2a study (BALANCED).[13] With a two-sided Pearson χ^2 test for proportion difference (significance level 0·05), 36 patients per group completing the study would provide at least 95% power to detect a 40% difference between the placebo and each active group.

The full analysis set (FAS) included all patients who were randomised into the phase 2b study. The liver biopsy analysis set (LBAS), a subset of the FAS, was used for histology analyses, including the primary efficacy analysis, and included all patients with baseline and week 24 liver biopsies. The safety set included all patients who received at least one dose of study drug.

All other biomarkers, including non-invasive markers of fibrosis, were assessed in the FAS using data from patients with non-missing values, without performing any imputations.

The prespecified primary efficacy analysis was done in the LBAS to estimate treatment effect under ideal conditions (completer analysis). The Cochran-Mantel-Haenszel test was used to compare differences between efruxifermin and placebo groups based on proportion of patients who met the primary histology endpoint, adjusting for the stratification factors of type 2 diabetes presence and baseline fibrosis stage. The point estimates and 95% CIs were constructed using the Miettinen and Nurminen method. For each treatment group versus placebo, the risk ratios (RRs) and corresponding 95% CIs were calculated. The primary efficacy endpoint was tested at a type I error rate of 0·05 (two-sided), without any adjustment for multiplicity. The p values associated with secondary and exploratory endpoints are therefore considered nominal. A prespecified sensitivity analysis also evaluated the primary and key secondary histology endpoints in the FAS, in which missing post-baseline biopsy results were imputed as non-response (intent-to-treat analysis). The primary endpoint was evaluated in prespecified subgroups based on variables of interest, such as presence of type 2 diabetes or use of statins at baseline, and post-hoc analyses evaluated the primary endpoint by GLP-1 use at baseline.

A Cochran-Mantel-Haenszel test was used for analysis of responders for other histology endpoints and for the proportion of patients who normalised HFF. Point estimates and 95% CIs were calculated for the differences in proportions. Analysis of covariance (ANCOVA) was used to determine changes from baseline to week 24 in HFF and liver stiffness with baseline as a covariate and controlling for stratification factors. For other endpoints, including markers of liver injury and fibrosis, lipid and glucose metabolism, and bodyweight, least-squares mean changes from baseline were analysed using mixed-model repeated-measures using baseline as a covariate and controlling for stratification factors, and presented as least-squares means with 95% CIs and p values. All data processing, summarisation, and analyses were performed using SAS (version 9.4 or later). The association between normalisation of liver fat or ALT and AST normalisation with histological improvements was evaluated in a post-hoc analysis calculated from a two-sided Fisher's exact test using GraphPad Prism (version 10.0.0). An external data monitoring committee, comprising two hepatologists, a cardiologist, and a statistician, reviewed the progress of the study.

This trial is registered with ClinicalTrials.gov, NCT04767529.

	Placebo	Efruxifermin 28 mg	Efruxifermin 50 mg
Changes in HFF			
Number of patients with data on HFF	42	38	35
Least-squares mean (SE) relative percentage change	−6·0 (4·0)	−51·6 (4·3)	−63·7 (4·4)
95% CI	(−14·0 to 1·9)	(−60·1 to −43·0)	(−72·4 to −54·9)
p value	0·14	<0·001	<0·001
Treatment comparison of percentage change from baseline (efruxifermin – placebo)			
Least-squares mean (SE)	..	−45·5 (5·6)	−57·6 (5·7)
95% CI	..	(−56·6 to −34·4)	(−68·9 to −46·4)
p value	..	<0·001	<0·001
Number (proportion) of patients with ≥30% relative reduction	9 (21%)	32 (84%)	31 (89%)
Percentage point difference from placebo (efruxifermin – placebo)	..	62·2	67·7
95% CI	..	(44·7–79·6)	(51·4– 83·9)
p value	..	<0·001	<0·001
Number (proportion) of patients with ≥50% relative reduction	1 (2%)	24 (63%)	27 (77%)
Percentage point difference from placebo (efruxifermin – placebo)	..	59·6	74·1
95% CI	..	(43·2 to 76·1)	(59·2 to 89·1)
p value	..	<0·001	<0·001
Number (proportion) of patients with normalised liver fat (≤5%)	1 (2%)	13 (34%)	18 (51%)
Percentage difference from placebo (efruxifermin – placebo)	..	31·3	50·4
95% CI	..	(15·3 to 47·3)	(33·4 to 67·4)
p value	..	<0·001	<0·001
Markers of whole-body metabolic health			
Number of patients with data on triglycerides	42	35	35
Least-squares mean absolute change (SE), mg/dL	10·4 (6·7)	−41·7 (7·2)	−47·0 (7·2)
95% CI	(−2·9 to 23·7)	(−56·0 to −27·4)	(−61·2 to −32·7)
p value	0·125	<0·001	<0·001
Treatment comparison of change from baseline (efruxifermin – placebo)			
Least-squares mean (SE), mg/dL	..	−52·1 (9·8)	−57·4 (9·7)
95% CI	..	(−71·4 to −32·8)	(−76·6 to −38·1)
p value	..	<0·001	<0·001
Number of patients with data on HDL cholesterol	42	35	35
Least-squares mean (SE), mg/dL	−1·1 (1·1)	10·1 (1·2)	11·4 (1·2)
95% CI	(−3·3 to 1·1)	(7·7 to 12·4)	(9·0 to 13·7)
p value	0·310	<0·001	<0·001
Treatment comparison of change from baseline (efruxifermin – placebo)			
Least-squares mean (SE), mg/dL	..	11·2 (1·6)	12·5 (1·6)
95% CI	..	(8·0 to 14·3)	(9·3 to 15·6)
p value	..	<0·001	<0·001

(Table 2 continues on next page)

Role of the funding source

The funder of the study had a role in the study design, data collection, data analysis, data interpretation, and writing of the report.

Results

Between March 22, 2021, and Feb 7, 2022, 128 patients (mean age 54·7 years [SD 10·4]; 79 [62%] women and 49 men [38%]; 118 [92%] white; and 56 [41%] Hispanic or

Latino) were randomly assigned to receive subcutaneous placebo (n=43), efruxifermin 28 mg (n=42), or efruxifermin 50 mg (n=43) once weekly for 24 weeks (FAS; figure 1). Two patients randomised to the efruxifermin 28 mg group did not receive the drug because of an administrative error and were considered early discontinuations. An additional 11 patients discontinued study treatment before week 24: three (7%) patients in the efruxifermin 28 mg group, four (9%) patients in the efruxifermin 50 mg, and one (2%) patient in the placebo group for administrative reasons; and two (5%) patients in the efruxifermin 28 mg group and three (7%) patients in the efruxifermin 50 mg group due to adverse events. Most (115 [90%]) patients completed 24 weeks of treatment in the study; biopsies were available at week 24 for 113 patients.

Demographic and clinical characteristics were mostly balanced among groups and consistent with patients at high risk for progressive NASH (table 1). Patients predominantly had type 2 diabetes (90 [70%]) and F3 fibrosis (84 [66%]). Distribution of select background medications by treatment group is shown in table 1. At screening, GLP-1 receptor agonists we used by 16 (13%) patients and SGLT2 inhibitors were used by 19 (15%) patients, and maintained at a stable dose for a minimum of 3 months before collection of biopsies.

In the LBAS population, fibrosis improved without worsening of NASH in significantly higher proportions of patients in the efruxifermin groups versus the placebo group: 15 (39%) of 38 patients in the 28 mg efruxifermin group (RR 2·3 [95% CI 1·1–4·8] *vs* placebo; p=0·025) and 14 (41%) of 34 patients in the 50 mg efruxifermin group (2·2 [1·0–5·0] *vs* placebo; p=0·036) met the endpoint versus eight (20%) of 41 patients in the placebo group (figure 2). A sensitivity analysis based on the FAS, including 15 patients who missed a biopsy being imputed as no improvement in fibrosis, found that more patients had an improvement in fibrosis without worsening of NASH in the efruxifermin 28 mg group (15 [36%] of 42 patients) than in the placebo group (eight [19%] of 43 patients; RR 2·2 [95% CI 1·0–4·8]; p=0·033). The difference (15%; RR 1·9 [95% CI 0·8–4·3]; p=0·123) in the proportion of patients who reached this endpoint was not significant for the 50 mg efruxifermin group compared with the placebo group. Results of the primary endpoint were comparable in the subgroup of patients with type 2 diabetes, in patients not taking GLP-1 receptor agonists at baseline, and were unrelated to statin use at baseline (appendix p 15). In the LBAS, 15 (39%) patients in the efruxifermin 28 mg group and 14 (41%) patients in the efruxifermin 50 mg group achieved the secondary endpoint of improvement in liver fibrosis (by ≥1 stage in NASH-CRN fibrosis score), regardless of NASH worsening, compared with nine (22%) patients in the placebo group (difference in proportions *vs* placebo 20% [95% CI 0·4 to 39·5]; p=0·053 for 28 mg; 20% [–1·5% to 41·2]; p=0·069 for 50 mg). Compared with the primary endpoint, one additional patient in the placebo group had fibrosis improvement, but was not deemed to be a responder in the primary endpoint due to worsening of NASH. All patients treated with efruxifermin who achieved improvement in fibrosis also had an improvement in NASH.

In the LBAS population, NASH resolved without fibrosis worsening in 18 (47%) patients in the

	Placebo	Efruxifermin 28 mg	Efruxifermin 50 mg
(Continued from previous page)			
Number of patients with data on non-HDL cholesterol	42	35	35
Least-squares mean (SE), mg/dL	5·3 (3·8)	–17·7 (4·1)	–18·5 (4·1)
95% CI	(–2·2 to 12·8)	(–25·7 to –9·6)	(–26·5 to –10·4)
p value	0·167	<0·001	<0·001
Treatment comparison of change from baseline (efruxifermin – placebo)			
Least-squares mean (SE), mg/dL	..	–23·0 (5·5)	–23·7 (5·5)
95% CI	..	(–33·8 to –12·1)	(–34·6 to –12·9)
p value	..	<0·001	<0·001
Number of patients with data on LDL cholesterol	42	35	35
Least-squares mean (SE), mg/dL	3·2 (3·5)	–11·2 (3·7)	–11·1 (3·7)
95% CI	(–3·7 to 10·1)	(–18·5 to –3·8)	(–18·4 to –3·7)
p value	0·358	0·003	0·003
Treatment comparison of change from baseline (efruxifermin – placebo)			
Least-squares mean (SE), mg/dL	..	–14·4 (5·0)	–14·3 (5·0)
95% CI	..	(–24·3 to –4·5)	(–24·2 to –4·4)
p value	..	0·005	0·005
Number of patients with data on glycated haemoglobin A_{1c} %	42	37	36
Least-squares mean (SE)	0·0 (0·1)	–0·3 (0·1)	–0·4 (0·1)
95% CI	(–0·3 to 0·2)	(–0·6 to –0·1)	(–0·7 to –0·2)
p value	0·865	0·014	<0·001
Treatment comparison of change from baseline (efruxifermin – placebo)			
Least-squares mean (SE)	..	–0·3 (0·2)	–0·4 (0·2)
95% CI	..	(–0·6 to 0·1)	(–0·8 to –0·1)
p value	..	0·095	0·014
Number of patients with data on HOMA-IR	39	32	33
Least-squares mean (SE)	0·9 (1·2)	–5·0 (1·3)	–5·2 (1·3)
95% CI	(–1·5 to 3·3)	(–7·5 to –2·4)	(–7·6 to –2·7)
p value	0·457	<0·001	<0·001
Treatment comparison of change from baseline (efruxifermin – placebo)			
Least-squares mean (SE)	..	–5·9 (1·7)	–6·1 (1·7)
95% CI	..	(–9·2 to –2·5)	(–9·4 to –2·7)
p value	..	<0·001	<0·001
Number of patients with data on C-peptide	41	37	35
Least-squares mean (SE), µg/L	0·1 (0·2)	–0·7 (0·2)	–1·0 (0·2)
95% CI	(–0·3 to 0·4)	(–1·1 to –0·4)	(–1·3 to –0·6)
p value	0·691	<0·001	<0·001
Treatment comparison of change from baseline (efruxifermin – placebo)			
Least-squares mean (SE), µg/L	..	–0·8 (0·3)	–1·0 (0·3)
95% CI	..	(–1·3 to –0·3)	(–1·5 to –0·5)
p value	..	0·002	<0·001

(Table 2 continues on next page)

efruxifermin 28 mg group (RR 3·1 [95% CI 1·4–6·9] *vs* placebo; p=0·002) and 26 (76%) patients in the efruxifermin 50 mg group (5·2 [2·4–11·1] *vs* placebo; p<0·001), compared with six (15%) patients in the placebo group (figure 2). For the corresponding prespecified sensitivity analysis in the FAS, NASH resolution was reported in 18 (43%) patients in the efruxifermin 28 mg group (RR 2·9 [95% CI 1·3–6·6] *vs* placebo; p=0·005) and 26 (60%) patients in the efruxifermin 50 mg group (4·2 [2·0–9·1] *vs* placebo; p<0·001), compared with six (14%) patients in the placebo group. Consistent with efruxifermin's effects on NASH resolution and regression of fibrosis, higher proportions of patients in the efruxifermin groups reached the exploratory composite endpoint of NASH resolution and fibrosis improvement compared with patients in the placebo group (figure 2). A higher proportion of patients in the efruxifermin groups also reached the exploratory endpoint of fibrosis improvement of at least 2 stages with no worsening of NASH compared with those in the placebo group (figure 2).

Effects of efruxifermin on liver fat content were assessed as secondary (least squares mean change from baseline in relative reduction of HFF) or exploratory (proportions of patients reaching ≥50% relative reduction or normalisation of liver fat) endpoints. Least-squares mean (SE) relative percentage change in HFF was –51·6 (SE 4·3) and –63·7 (4·4) with efruxifermin at week 24 in the 28 mg and 50 mg groups, respectively, compared with –6·0 (4·0) in the placebo group (table 2). Most patients in the efruxifermin groups had at least 50% relative reductions in HFF. Liver fat was normalised (≤5%) in 13 (34%) patients in the efruxifermin 28 mg group and 18 (51%) patients in the efruxifermin 50 mg group, compared with one (2%) patient in the placebo group (table 2).

Among additional secondary endpoints at 24 weeks, participants receiving efruxifermin had dose-related improvements in markers of liver injury (figure 3A–D). In a post-hoc analysis of patients with high ALT at baseline, concentrations were normalised in nine (28%) of 32 patients in the efruxifermin 28 mg group and 17 (50%) of 34 patients in the efruxifermin 50 mg group, compared with two (5%) of 40 patients in the placebo group. Among participants with high AST at baseline, concentrations were normalised in 18 (67%) of 27 patients in the efruxifermin 28 mg group and 22 (85%) of 26 patients in the efruxifermin 50 mg group, compared with four (14%) of 29 patients in the placebo group. In post-hoc analysis, normalisation of ALT and AST was associated with increased odds of reaching NASH resolution with no worsening of fibrosis among patients treated with efruxifermin (appendix p 8).

For other secondary endpoints, urate—a marker of hepatocyte ATP depletion and oxidative stress—had a placebo-corrected least-squares mean change from baseline of –0·7 mg/dL (SE 0·2; p=0·002) in the efruxifermin 28 mg group and –0·5 mg/dL (0·2; p=0·011) in the efruxifermin 50 mg group (table 2). Serum biomarkers of soft-tissue fibrosis (ELF score) and fibrogenesis (Pro-C3) decreased in the efruxifermin

	Placebo	Efruxifermin 28 mg	Efruxifermin 50 mg
(Continued from previous page)			
Number of patients with data on adiponectin	42	36	33
Least-squares mean (SE), ng /mL	282·4 (316·9)	1413·4 (338·6)	3028·2 (344·7)
95% CI	(–345·1 to 910·0)	(742·8 to 2084·0)	(2345·6 to 3710·8)
p value	0·375	<0·001	<0·001
Treatment comparison of change from baseline (efruxifermin – placebo)			
Least-squares mean (SE), ng/mL	..	1131·0 (449·9)	2745·8 (454·4)
95% CI	..	(239·8 to 2022·1)	(1845·7 to 3645·8)
p value	..	0·013	<0·001
Number of patients with data on urate	42	36	36
Least-squares mean (SE), mg/dL	0·0 (0·2)	–0·6 (0·2)	–0·5 (0·2)
95% CI	(–0·3 to 0·3)	(0·9 to –0·3)	(–0·8 to –0·2)
p value	0·792	<0·001	0·001
Treatment comparison of change from baseline (efruxifermin – placebo)			
Least-squares mean (SE), mg/dL	..	–0·7 (0·2)	–0·5 (0·2)
95% CI	..	(–1·1 to –0·2)	(–1·0 to –0·1)
p value	..	0·002	0·011
Changes in bodyweight			
Number of patients with data on bodyweight	42	37	36
Least-squares mean (SE), kg	–0·6 (0·8)	–0·2 (0·9)	–2·9 (0·9)
95% CI	(–2·2 to 1·0)	(–1·9 to 1·5)	(–4·6 to –1·2)
p value	0·479	0·803	0·001
Treatment comparison of change from baseline (efruxifermin – placebo)			
Least-squares mean (SE), kg	..	0·4 (1·2)	–2·3 (1·2)
95% CI	..	(–2·0 to 2·7)	(–4·7 to 0·0)
p value	..	0·756	0·052
Changes in glycaemic control in patients with type 2 diabetes			
Number of patients with type 2 diabetes with data on glycated haemoglobin A_{1c} %	27	28	27
Least-squares mean (SE)	0·0 (0·2)	–0·5 (0·2)	–0·5 (0·12)
95% CI	(–0·3 to 0·3)	(–0·8 to –0·2)	(–0·9 to –0·2)
p value	0·963	0·004	0·001
Treatment comparison of change from baseline (efruxifermin – placebo)			
Least-squares mean (SE)	..	–0·5 (0·2)	–0·6 (0·2)
95% CI	..	(–1·0 to 0·0)	(–1·0 to –0·1)
p value	..	0·041	0·021
Number of patients with type 2 diabetes with data on HOMA-IR	25	26	26
Least-squares mean (SE)	2·2 (1·7)	–6·0 (1·7)	–6·1 (1·6)
95% CI	(–1·1 to 5·6)	(–9·4 to –2·7)	(–9·3 to –2·8)
p value	0·190	<0·001	<0·001
Treatment comparison of change from baseline (efruxifermin – placebo)			
Least-squares mean (SE)	..	–8·2 (2·3)	–8·3 (2·3)
95% CI	..	(–12·9 to –3·6)	(–12·8 to –3·7)
p value	..	<0·001	<0·001

(Table 2 continues on next page)

groups but not placebo groups (figure 3E–F and appendix p 9). These improvements were associated with reductions in all components of the ELF score (appendix p 9). The placebo-corrected least-squares mean reduction in liver stiffness was 1·9 kPa (95% CI –4·3 to 0·6; p=0·131) in the efruxifermin 28 mg group and 3·6 kPa (–6·1 to –1·1; p=0·005) in the efruxifermin 50 mg group (figure 3G). NIS-4, a biomarker of risk of NASH, was significantly reduced from baseline by efruxifermin compared with placebo (appendix p 9).

Efruxifermin also improved systemic markers of whole-body lipid and glucose metabolism. Concentrations of triglyceride, non-HDL cholesterol, and LDL cholesterol were reduced after 24 weeks efruxifermin (table 2). Consistent with the reduction in triglyceride-rich lipoproteins, concentrations of apolipoproteins B and C3 were numerically lower (appendix p 11), whereas the concentration of HDL cholesterol increased.

HOMA-IR and concentrations of C-peptide decreased in both efruxifermin groups compared with placebo (table 2). In the overall study cohort HbA_{1c} was reduced by efruxifermin but these reductions were significant only in the 50 mg group. In patients with type 2 diabetes who continued on their baseline antidiabetic medications, the least-squares mean change from baseline versus placebo comparison was –0·5 (95% CI –1·0 to 0·0; p=0·041) for the 28 mg efruxifermin group and –0·6 (–1·0 to –0·01; p=0·021) for the 50 mg group. Adiponectin concentrations were substantially increased in the efruxifermin groups.

Although the efruxifermin 50 mg group had a least-squares mean change from baseline in bodyweight of –2·9 kg (SE 0·9; p=0·001), this was not significantly different from placebo (least-squares mean difference –2·3 kg (95% CI –4·7 to 0·0; p=0·052; table 2).

116 (92%) patients had at least one treatment-emergent adverse event (TEAE), most of which were grade 1–2 (table 3). The most frequently reported efruxifermin-related TEAEs were diarrhoea (14 [35%] patients in the 28 mg group and 14 [33%] patients in the 50 mg group *vs* six [14%] patients in the placebo group; all except one event grade 1–2) and nausea (10 [25%] patients in the 28 mg group and 14 [33%] patients in the 50 mg group *vs* five [12%] patients in the placebo group; all grade 1–2). No deaths occurred.

Four serious adverse events occurred in four patients in the efruxifermin 50 mg group, including one event of ulcerative esophagitis in a patient with a history of gastroesophageal reflux disease that was considered by the investigator to be drug related. The three other events were deemed by the investigator to be unrelated to efruxifermin: acute necrotising pancreatitis in a patient with multiple risk factors for pancreatitis at baseline, facial oedema, and hospitalisation for COVID-19. In addition, one drug-related, non-serious adverse event of diarrhoea, in the efruxifermin 50 mg group, was classified as grade 3.

Five patients discontinued before week 24 due to an adverse event (table 3): two in the efruxifermin 28 mg group (both deemed by the investigator to be drug related) and three in the efruxifermin 50 mg group (two of which were deemed drug related).

No clinically significant, dose-dependent changes, compared with placebo, were identified based on laboratory parameters or electrocardiograms. Neither respiratory rate nor heart rate changed for both doses of efruxifermin. Although transiently higher systolic blood pressure was noted during the first 4 weeks of treatment for patients receiving efruxifermin 28 mg, a dose response was not evident, and change from baseline at week 24 was not significantly different from placebo for either dose (appendix p 12).

In patients who received at least one dose of efruxifermin (safety set), efruxifermin was associated with a significant increase in a marker of bone resorption, CTX-1. There was also a significant decrease in procollagen I N-terminal pro-peptide (P1NP), indicating reduced synthesis of type-I collagen.[16] The concentration of P1NP at baseline correlated with that of procollagen type III N-terminal peptide (P3NP), a marker of soft-tissue fibrogenesis (appendix p 19).[17] Changes in P1NP from baseline to week 24 also correlated with changes in P3NP (appendix p 19). When analysed by demographic subgroup based on sex and age (as an approximate indicator of menopausal status), the values for CTX-1 and P1NP remained within their respective reference ranges. In this context, there was an imbalance in use of vitamin D supplements at baseline, with 25 (58%) of 43 patients taking them in the placebo group, compared with only 13 (33%) of 40 patients

	Placebo	Efruxifermin 28 mg	Efruxifermin 50 mg
(Continued from previous page)			
Number of patients with type 2 diabetes with data on C-peptide	26	28	26
Least-squares mean (SE), µg/L	0·2 (0·2)	–0·8 (0·2)	–1·0 (0·2)
95% CI	(–0·3 to 0·6)	(–1·3 to –0·3)	(–1·5 to –0·5)
p value	0·501	<0·001	<0·001
Treatment comparison of change from baseline (efruxifermin – placebo)			
Least-squares mean (SE), ug/L	..	–1·0 (0·3)	–1·1 (0·3)
95% CI	..	(–1·6 to –0·3)	(–1·8 to –0·5)
p value	..	0·004	<0·001
Number of patients with type 2 diabetes with data on adiponectin	27	27	25
Least-squares mean (SE), ng/mL	167·4 (357·5)	1233·0 (352·3)	2704·4 (353·9)
95% CI	(–543·9 to 878·7)	(532·0 to 1933·9)	(2000·3 to 3408·4)
p value	0·641	<0·001	<0·001
Treatment comparison of change from baseline (efruxifermin – placebo)			
Least-squares mean (SE), ng/mL	..	1065·5 (489·0)	2536·9 (490·4)
95% CI	..	(92·7 to 2038·4)	(1561·1 to 3512·7)
p value	..	0·032	<0·001

Data are presented in all patients (full analysis set) with available (non-missing) baseline and on-treatment values for MRI-PDFF and biomarkers; no imputations were performed for missing values. HHF=hepatic fat fraction.

Table 2: **Changes in HFF, metabolic markers, and bodyweight from baseline to week 24**

in the efruxifermin 28 mg and 15 (35%) of 43 patients in the efruxifermin 50 mg groups.

Biomarkers of liver function and haemostasis were generally stable during the study (appendix p 14).

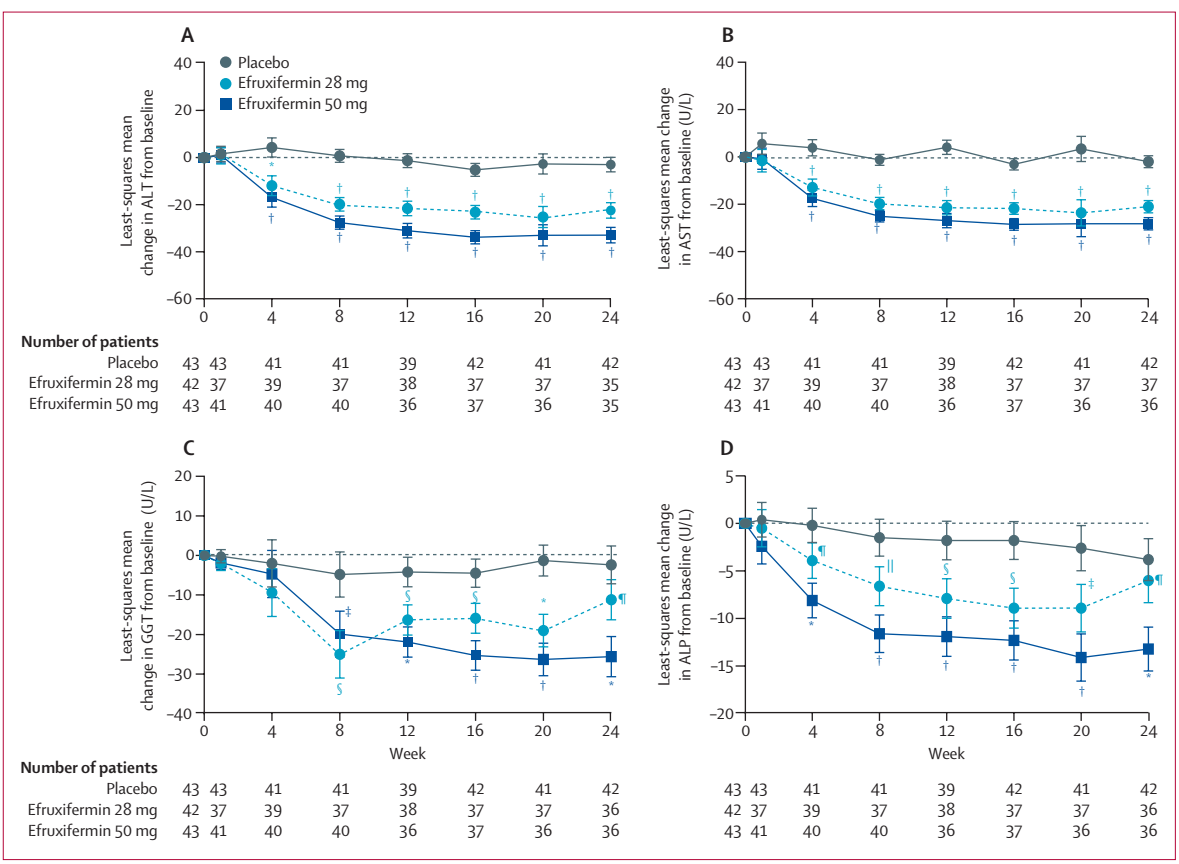
In patients who received at least one dose of efruxifermin, 65 (83%) of 78 patients were positive for treatment-emergent ADA. The titres of ADA were low, as noted in previous studies,[13] and developed slowly, over 16–24 weeks. Presence of ADA did not appear to alter the magnitude of pharmacodynamic response, indicated by changes in serum levels of adiponectin and triglycerides (appendix p 16).

Discussion

In this randomised controlled trial of patients with biopsy-confirmed NASH and F2 or F3 fibrosis, efruxifermin improved fibrosis and resolved NASH in a significantly higher proportion of patients compared with placebo, resulting in histological improvements that are reasonably likely to predict clinical benefit. The primary efficacy analysis was based on the LBAS (completer set) to estimate the treatment effect under ideal conditions. The primary endpoint of significant improvement in fibrosis was met. Of note, resolution of NASH and regression of fibrosis was observed in approximately a third of patients treated with efruxifermin. This composite endpoint is the most clinically meaningful indication of improved liver health since it reflects underlying disease activity (steatohepatitis) and the sequela of active collagen deposition (fibrosis). These data have been used for dose selection in future trials. A phase 3 trial is planned to confirm these findings. Participants in the phase 2b HARMONY study will continue in their randomly assigned groups for 96 weeks, allowing for further collection of data on safety, tolerability, and durability of histologic response.

Consistent with these histopathological improvements, efruxifermin rapidly (within 4 weeks) reduced established markers of liver injury, with high rates of normalisation of ALT and AST by 24 weeks. Concurrently, markers of collagen synthesis and fibrosis were reduced to an extent associated with 1-stage reversal of fibrosis.[14,17,18]

Underlying these broad improvements in liver health is a large reduction in hepatic steatosis. Post-hoc analyses of data from BALANCED and this study (HARMONY) revealed higher odds of NASH resolution among patients receiving efruxifermin whose levels of liver fat normalised, compared with patients whose levels remained higher than 5%.[19] A major contributor to the normalisation of liver fat by efruxifermin appears to be inhibition of adipose tissue lipolysis, since approximately half the hepatic triglyceride flux derives from uptake of free fatty acids released by adipose tissue in patients with NASH.[20]



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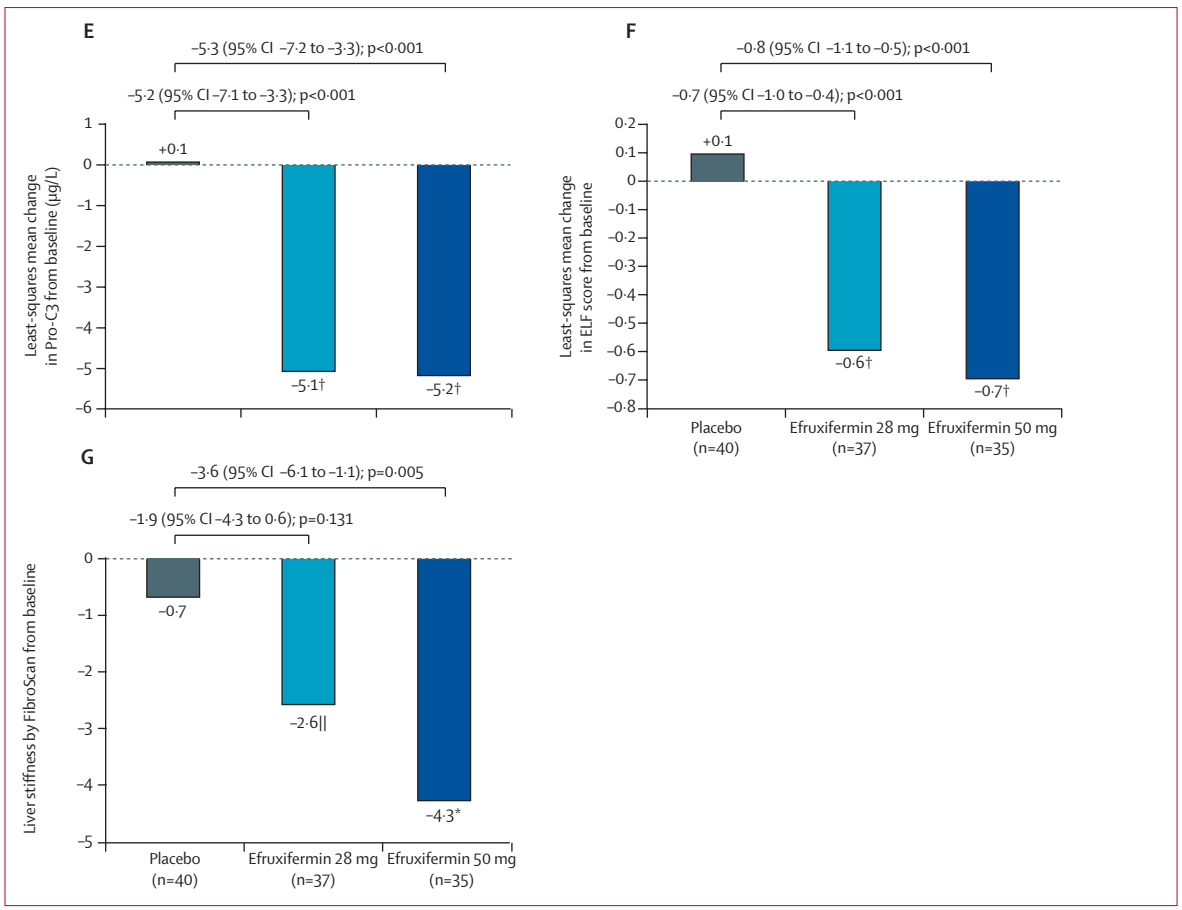

Figure 3: **Change at week 24 from baseline in markers of liver injury and fibrosis**
Least-squares mean (SE) changes from baseline for ALT (A), AST (B), GGT (C), and ALP (D), using the full analysis set (mixed-model repeated-measures). Least-squares mean (SE) changes from baseline and treatment comparison with placebo-corrected least-squares mean change (95% CI) for pro-C3 (E), ELF score (F), and liver stiffness by FibroScan (G), using the full analysis set (mixed-model repeated-measures for Pro-C3 and ELF Score, ANCOVA for liver stiffness). For all markers, only patients with non-missing values were included and no imputations were performed for missing values. ALP=alkaline phosphatase. ALT=alanine aminotransferase. AST=alanine aminotransferase. ELF=enhanced liver fibrosis. GGT=gamma glutamyl transferase. Pro-C3=N-terminal type-III collagen pro-peptide. *p<0·01 *vs* placebo. †p<0·001 *vs* placebo. ‡p<0·001 *vs* baseline. §p<0·05 *vs* placebo. ¶p<0·05 *vs* baseline. ||p<0·01 *vs* baseline.

Efruxifermin-mediated improvements in adipose tissue insulin sensitivity likely underlie the suppression of lipolysis. Agonists of peroxisome proliferator activator receptor gamma or GLP-1 receptor also suppress lipolysis, by enhancing insulin's action on adipose tissue, but appear to reduce liver fat to a lesser extent than efruxifermin.[21,22] We propose that efruxifermin also directly inhibits hepatic de-novo lipogenesis, the other major contributor to liver triglycerides,[23] thereby contributing to normalisation of liver fat. Of the FGF21 receptors, FGFR2c and FGFR3c seem likely to mediate these direct effects on liver.[24] For maximal reduction in liver fat, FGF21 analogues should, therefore, activate not only FGFR1c in adipocytes but also FGFR2c and FGFR3c in hepatocytes.

Loss of at least 10% of bodyweight after bariatric surgery or lifestyle modification reduces steatosis and can resolve steatohepatitis within 1 year.[25] However, meaningful rates of fibrosis reversal require considerably longer timespans (up to 5 years).[26] The regression of fibrosis within 24 weeks, independent of large reductions in bodyweight, indicates that efruxifermin accelerates net fibrolysis, potentially as a result of inhibiting fibrogenesis.[14,23] The observation that markers of fibrogenesis and hepatocyte injury appear to decrease concurrently with decreasing liver fat, rather than after normalisation of liver fat, suggests that efruxifermin simultaneously activates pathways that protect against intracellular stressors, to suppress hepatocyte death or dedifferentiation, while reducing fibrogenesis and fibrosis directly. We propose therefore that rapid reversal of fibrosis within 24 weeks is due to efruxifermin simultaneously suppressing proinflammatory signalling, induced by death or dedifferentiation of hepatocytes, and directly inhibiting differentiation of hepatic stellate cells into collagen-secreting myofibroblasts.[2] An absence of correlation between extent of reduction in liver fat and regression of fibrosis is consistent with our hypothesis that the decline in collagen synthesis during the first 24 weeks of treatment does not depend entirely on clearance of liver

	Placebo (n=43)	Efruxifermin 28 mg (n=40)	Efruxifermin 50 mg (n=43)	Total (n=126)
Any TEAEs with an incidence >10% in any group	21 (49%)	29 (73%)	34 (79%)	84 (67%)
Diarrhoea	8 (19%)	16 (40%)	17 (40%)	41 (33%)
Nausea	10 (23%)	11 (28%)	18 (42%)	39 (31%)
Injection site erythema	8 (19%)	8 (20%)	8 (20%)	24 (19%)
Increased appetite	2 (5%)	8 (20%)	10 (23%)	20 (16%)
COVID-19	3 (7%)	4 (10%)	9 (21%)	16 (13%)
Headache	5 (12%)	6 (15%)	5 (12%)	16 (13%)
Vomiting	4 (9%)	6 (15%)	6 (14%)	16 (13%)
Injection site bruising	2 (5%)	7 (18%)	4 (9%)	13 (10%)
Frequent bowel movements	1 (2%)	9 (23%)	0	10 (8%)
Injection site pruritus	1 (2%)	3 (8%)	6 (14%)	10 (8%)
Injection site rash	1 (2%)	3 (8%)	6 (14%)	10 (8%)
Any TEAEs (maximum severity grade)	38 (88%)	36 (90%)	42 (98%)	116 (92%)
Grade 1	13 (30%)	12 (30%)	13 (30%)	38 (30%)
Grade 2	25 (58%)	23 (58%)	25 (58%)	73 (58%)
Grade 3	0	1 (3%)	4 (9%)	5 (4%)
Grade 4	0	0	0	0
Grade 5	0	0	0	0
Drug-related TEAEs with an incidence >10% in any group	21 (49%)	30 (75%)	35 (81%)	86 (68%)
Diarrhoea	6 (14%)	14 (35%)	14 (33%)	34 (27%)
Nausea	5 (12%)	10 (25%)	14 (33%)	29 (23%)
Increased appetite	2 (5%)	7 (18%)	10 (23%)	19 (15%)
Frequent bowl movements	1 (2%)	8 (20%)	0	9 (7%)
Injection site erythema	5 (12%)	6 (15%)	7 (16%)	18 (14%)
Injection site bruising	1 (2%)	6 (15%)	3 (7%)	10 (8%)
Injection site rash	1 (2%)	3 (8%)	6 (14%)	10 (8%)
Injection site pruritus	1 (2%)	2 (5%)	6 (14%)	9 (7%)
TEAE leading to study drug discontinuation	0	2 (5%)	3 (7%)	5 (4%)
Diarrhoea*	0	1 (3%)	0	1 (1%)
Nausea*	0	0	1 (2%)	1 (1%)
Oesophagitis, ulcerative*	0	0	1 (2%)	1 (1%)
Vomiting*	0	0	1 (2%)	1 (1%)
Lymphadenopathy†	0	0	1 (2%)	1 (1%)
Weight increased*	0	1 (3%)	0	1 (1%)
Increased appetite*	0	1 (3%)	0	1 (1%)
Any treatment-emergent serious adverse events	0	0	4 (9%)	4 (3·2)
Oesophagitis, ulcerative*	0	0	1 (2%)	1 (1%)
Pancreatitis, necrotising†	0	0	1 (2%)	1 (1%)
Face oedema†	0	0	1 (2%)	1 (1%)
COVID-19†	0	0	1 (2%)	1 (1%)

Data are n (%). TEAE=treatment-emergent adverse event. *Related to study drug by the investigator. †Unrelated to study drug by the investigator.

Table 3: **Common TEAEs (safety set)**

fat. Longer term treatment might show an association of fibrosis improvement with normalisation of liver fat.

Cross-study comparisons are of limited value owing to heterogeneity in study design, conduct, and analysis, and enrolled patient populations. Nonetheless, the magnitude of placebo-adjusted improvement in fibrosis with efruxifermin is similar to that of 24 weeks treatment with another FGF21 analogue, pegozafermin, and with the PPAR agonist lanifibranor, and greater than observed with either semaglutide or resmetirom, in phase 2b studies, despite their longer duration of treatment.[27–29] However, the extent of NASH resolution with no worsening of fibrosis appears to be higher for efruxifermin, compared with corresponding response rates reported from other phase 2b clinical studies.[27–30] The placebo-adjusted response rates for 50 mg efruxifermin, of 28% in the FAS and 36% in the LBAS, for the combined endpoint of fibrosis improvement and NASH resolution are higher than those from other phase 2 trials reporting this endpoint. The response rates with efruxifermin for resolution of NASH without worsening of fibrosis, and for the combined endpoint of NASH resolution and improvement in fibrosis, are greater than those reported for the FGF21 analogues pegbelfermin and pegozafermin.[30,31] Pegbelfermin appears to be weakly active on adipose tissue, eliciting minimal and transient increases in adiponectin.[30] As a consequence, liver fat was modestly reduced, indicated by smaller reductions in markers of liver injury and fibrosis. Reductions in HFF and serum triglycerides in patients with NASH and F2 or F3 reported for pegozafermin at the highest dose tested (30 mg once a week)[31] appear to be smaller than those for once-weekly efruxifermin 50 mg.

Amelioration of dyslipidaemia and insulin resistance with efruxifermin would be expected to establish a healthier metabolic environment for the liver. As such, we propose that resolution of NASH and fibrosis is likely to be at least sustained, and potentially improved, with a longer period of treatment. Additionally, improved lipoprotein profiles indicate the potential of efruxifermin to reduce cardiovascular disease, the leading cause of mortality in patients with pre-cirrhotic NASH.[32,33]

The overall tolerability and safety profile of efruxifermin appeared acceptable. Both doses of efruxifermin were associated with more frequent gastrointestinal adverse events, mostly mild or moderate (98% grade 1 or 2), and transient. Only four patients (two in each efruxifermin dose group) discontinued due to drug-related adverse events, and one additional patient in the efruxifermin 50 mg group discontinued due to an unrelated adverse event, before week 24. The overall tolerability profile of efruxifermin at both doses appears comparable to that reported for other FGF21 analogues.[31,34]

Although the incidence of ADA was high, the analytical method is 10-fold more sensitive than regulatory guidance.[35] Pharmacodynamic responses to efruxifermin did not appear to be affected by presence of ADAs.

Although changes in bone biomarkers are consistent with a shift toward bone resorption, they might have been confounded by several factors. While P1NP is considered to be a marker of bone deposition, it has also been reported to be affected by changes in soft-tissue fibrosis.[16]

Inhibition of collagen synthesis in the liver might have contributed to the lower P1NP concentrations with efruxifermin treatment. Additionally, fatty liver disease has been associated with a deficiency in vitamin D.[36] In this study, an imbalance in use of vitamin D supplements between placebo and efruxifermin groups at baseline might have been a confounding factor. The relationship between these markers of bone turnover and bone mineral density will be monitored in the planned phase 3 study. Strengths of this study include its randomised, controlled design; adoption of a blinded, multi-reader, consensus-reading methodology for liver biopsies; enrolment of only patients with F2 or F3 fibrosis; and measurement of biomarkers of metabolic status, tissue injury, and fibrosis.

Limitations of the study include a relatively short duration for the primary endpoint, small sample size that was not adequately powered for an intent-to-treat analysis or a thorough evaluation of subgroups, limited ethnic representation, and absence of NASH not associated with obesity. The study is ongoing in a blinded manner, with patients being followed up for 96 weeks to evaluate longer term effects. Although the prespecified primary endpoint was met in the completer analysis for both efruxifermin groups compared with placebo, confirmation in a phase 3 study is required. Generalisability of the study results to patients with NASH who do not have type 2 diabetes or are not obese, as well as to patients already being treated with GLP-1 receptor agonists, remains to be confirmed by a phase 3 study.

In summary, efruxifermin appears to be a promising therapy for patients with fibrosis due to NASH. Acting across liver and adipose tissue, efruxifermin unburdens the liver of excess energy, thereby reducing liver fat and resolving NASH histopathology, while establishing a healthier whole-body metabolic environment that might support sustained resolution of steatohepatitis and fibrosis regression.

Contributors

The protocol was designed by KY, TR, RS, BdT, EF, AC, LS, and SAH. SAH, JPF, GN, GAA, KJL, WS, SG, MYS, CB, and PB contributed to data acquisition. The data were analysed by KY, TR, RS, BdT, EF, AC, DC, SAH, EJT, and LS. SAH, KY, and RS had access to all the data and can vouch for the integrity of the data analyses. The manuscript was written by SAH, RS, TR, KY, EJT, and DC. All authors reviewed the entire manuscript, had full access to all the data, and accept responsibility to submit for publication.

Declaration of interests

SAH receives grants or contracts from Axcella Health, BMS, Civi Biompharma, Conatus, Cymbay Therapeutics, Enyo Pharma SA, Galectin Therapeutics, Genentech, Genfit Corp, Gilead Sciences, Hepion Pharma, Hightide Therapeutics, Immuron, Intercept Pharma, Madriagal Pharma, NGM Biopharma, Northsea Therapeutics BV, Novartis Pharma, Novo Nordisk, Pfizer, Poxel, Sagimet Biosciences, Second Genome, and Viking Therapeutics. SAH receives consulting fees from Akero Therapeutics, AgomAB, Alentis Therapeutics, Aligos Thereapeutics, Alimentive, Altimmune, Axcella Health, Blade Therapeutics, Bluejay Therapeutics, Boston Pharmaceuticals, Boxer Capital, Canfite Biopharma, CIRIUS Therapeutics, CIVI Biopharma, CLDF, Cohbar, Corcept, Cymbay Therapeutics, Echosens North America, Enyo Pharma SA, Fibronostics, Foresite Labs, Fortress Biotech, Galectin Therapeutics, Galecto, Genfit, Gilead Sciences, GlaxoSmithKline, GNS Healthcare, GRI Bio, Hepagene Therapeutics, Hepta Bio, Hightide Therapeutics, Histoindex, Inipharm, Intercept Pharmaceuticals, Ionis, Kowa Research Institute, Madrigal Pharma, Medpace, Merck, MGMM Therapeutics, NGM Pharma, Northsea Therapeutics, Novartis Pharma, Novo Nordisk, Nutrasource, Perspectum Diagnostics, Pfizer, Piper Sandler & Co, Poxel, Prometic (now Liminal), Ridgeline Therapeutics, Sagimet Biosciences, Silverback Therapeutics, Sonic Incytes Medical, Terns, Viking Therapeutics, and Zafget. SAH is involved in clinical trials and/or receives support for attending meetings or travel from Akero Therapeutics, Axcella Health, Enyo Pharma, Galectin Therapeutics, Genfit Corp, Gilead Sciences, Hepion Pharma, Intercept Pharma, Ionis, Madrigal Pharma, NGM Biopharma, Poxel, Terns Inc, and Viking Therapeutics. SAH serves on advisory boards of Akero Therapeutics, Altimmune, Arrowhead Pharmaceuticals, Axcella Health, BVF Partners, Chronwell, Echosens North America, Foresite Labs, Galectin Therapeutics, Genfit, Gilead Sciences, Hepion Pharmaceuticals, Hightide Therapeutics, Histoindex, Humana, Intercept Pharmaceuticals, Madrigal Pharmaceuticals, Metacrine, NGM Biopharmaceuticals, Northsea Therapeutics, Novartis Pharmaceuticals, Novo Nordisk, Parthai, Poxel, Sagimet Biosciences, Sonic Incytes Medical, and Terns. JPF has received research support through his institution from Akero Therapeutics. His institution also receives grants or contracts from Eli Lilly, Novo Nordisk, Pfizer, Boehringer Ingelheim, Merck, 89BIO, Altimmune, Intercept, Madrigal, Carmot Therapeutics, NorthSea Therapeutics, and Novartis. JPF receives consulting fees from Akero Therapeutics, Altimmune, Boehringer Ingelheim, Carmot Therapeutics, Echosens, 89bio, Eli Lilly, Gilead, Merck, Novo Nordisk, Pfizer, and Sanofi. He has received honoraria or payment for lectures from Eli Lilly, Sanofi, Novo Nordisk, and Merck. He has received travel support from Eli Lilly, Novo Nordisk, and Sanofi. JPF participates on data safety monitoring or advisory boards of Altimmune, Eli Lilly, Novo Nordisk, Boehringer Ingelheim, Merck, Pfizer, and Sanofi. GN has received consulting fees from Boehringer Ingleheim, where he has also participated in advisory boards; he has payment or honoraria for lectures, presentations, or other activities from Intercept. WS received financial support for research through his organisation and travel to conferences from Akero Therapeutics. He receives research grants or contracts from Merck and Astra Zeneca. PB has received research hrough his organisation from Akero Therapeutics and consulting fees from 89Bio, Boehringer, Intercept, Ionis, Madrigal, Poxel, and Sagimet. CB has a service funding contract with Akero Therapeutics, Icon, Covance, Perspectum, Med pace, Hologic, and Roche-Ventana. She has received payment for lectures or presentations from Novo Nordisk and Acelabio. SG and MYS received funding through their organisations from Akero Therapeutics. DC, EF, BdT, RS, EJT, TR, AC, and KY are employees of Akero Therapeutics and hold stock and/or options in the company. GAA, KJL, and LS declare no competing interests.

Data sharing

At the time of this submission, the study remains ongoing and blinded, as a result of which patient-level data cannot be shared. Further, the datasets generated and analysed during the current study are commercially sensitive and are not publicly available. Requests for data supporting findings in the manuscript should be made to the corresponding author and will be reviewed individually. Data can be shared in the form of aggregated summaries. Individual patient-level raw data containing confidential or identifiable patient information is subject to patient privacy and cannot be shared.

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