Safety, pharmacokinetics, and pharmacodynamics of pegozafermin in patients with non-alcoholic steatohepatitis: a randomised, double-blind, placebo-controlled, phase 1b/2a multiple-ascending-dose study

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Summary

Background Management strategies for non-alcoholic steatohepatitis (NASH) are based predominantly on lifestyle modification, with no approved disease-modifying drugs yet available. We aimed to evaluate the safety, pharmacokinetics, and pharmacodynamics of pegozafermin (BIO89-100), a glycoPEGylated FGF21 analogue, in participants with NASH.

Methods This randomised, double-blind, placebo-controlled, phase 1b/2a multiple-ascending-dose study enrolled adults (aged 21–75 years) who had NASH with stage F1–F3 fibrosis, or non-alcoholic fatty liver disease and a high risk of NASH (referred to in this study as phenotypic NASH) due to central obesity with type 2 diabetes, or central obesity with increased alanine aminotransferase (ALT) or a Fibroscan score of 7 kPa or greater, across 12 specialist centres and clinics in the USA. Patients were centrally randomised by use of an interactive web response system to receive subcutaneously administered pegozafermin (3, 9, 18, or 27 mg once weekly; 18 or 36 mg once every 2 weeks) or placebo for 12 weeks. The primary endpoints were the safety, tolerability, and pharmacokinetics of pegozafermin. This trial is registered with ClinicalTrials.gov (NCT04048135).

Findings Between July 29, 2019, and Aug 3, 2020, 275 participants were screened and 81 (15 [19%] with biopsy-confirmed NASH) were randomly assigned: 62 to pegozafermin (six to 3 mg once weekly, 12 to 9 mg once weekly, 11 to 18 mg once weekly, ten to 27 mg once weekly, 14 to 18 mg once every 2 weeks, and nine to 36 mg once every 2 weeks) and 19 to placebo; 63 received pegozafermin and 18 received placebo, as one participant in the placebo group inadvertently received 3 mg pegozafermin once weekly. Adverse events were reported in eight (44%) of 18 participants in the pooled placebo group, six (86%) of seven in the 3 mg once weekly pegozafermin group, four (33%) of 12 in the 9 mg once weekly group, seven (64%) of 11 in the 18 mg once weekly group, seven (70%) of ten in the 27 mg once weekly group, eight (57%) of 14 in the 18 mg once every 2 weeks group, and eight (89%) of nine in the 36 mg once every 2 weeks group. The most common treatment-related adverse event was mild increased appetite (in ten [16%] of 63 participants in the pooled pegozafermin group *vs* none of 18 in the pooled placebo group), which was not associated with bodyweight gain. Two patients discontinued treatment due to an adverse event (one each in the 27 mg once weekly and 18 mg once every 2 weeks groups). No treatment-related serious adverse events or deaths occurred. Dose-proportional pharmacokinetics were observed. Anti-drug antibodies were detected in 41 (65%) of 63 participants treated with pegozafermin. By week 13, pegozafermin significantly reduced the least squares mean (LSM) absolute differences in hepatic fat fraction versus pooled placebo (–8·9% [95% CI –14·8 to –3·1; p=0·0032] for 3 mg once weekly, –11·5% [–16·1 to –6·9; p<0·0001] for 9 mg once weekly, –8·9% [–13·7 to –4·2; p=0·0004] for 18 mg once weekly, –14·9% [–20·1 to –9·7; p<0·0001] for 27 mg once weekly, –10·4% [–14·7 to –6·1; p<0·0001] for 18 mg once every 2 weeks, and –11·1% [–16·2 to –6·0; p<0·0001] for 36 mg once every 2 weeks). At week 13, significant LSM relative reductions versus pooled placebo in ALT were observed for pegozafermin 9 mg once weekly, 18 mg once weekly, 27 mg once weekly, and 36 mg once every 2 weeks. At week 13, significant LSM relative reductions versus pooled placebo in aspartate aminotransferase were observed for pegozafermin 3 mg once weekly, 27 mg once weekly, and 36 mg once every 2 weeks. Significant improvements were also observed with pegozafermin treatment for triglycerides (9 mg once weekly, 27 mg once weekly, and 18 mg once every 2 weeks), LDL-C (9 mg once weekly and 27 mg once weekly), HDL-C (3 mg once weekly and 18 mg once every 2 weeks), non-HDL-C (9 mg once weekly and 27 mg once weekly), adiponectin (all doses except for 36 mg once every 2 weeks), PRO-C3 (27 mg once weekly), and bodyweight (27 mg once weekly). Changes in insulin resistance and HbA_{1c} were not significant.

Interpretation Pegozafermin was generally well tolerated and associated with clinically meaningful reductions in liver fat, measures of liver function, and circulating lipids. Further evaluation of pegozafermin in individuals with NASH is warranted.

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Introduction

Non-alcoholic steatohepatitis (NASH), the progressive form of non-alcoholic fatty liver disease (NAFLD), is a chronic disease characterised by steatosis in at least 5% of hepatocytes, lobular inflammation, and hepatocyte ballooning, with or without fibrosis.[1] The current global prevalence of NASH is 1·5–6·5% in the general population; in the USA, the prevalence of NASH is expected to increase from 16·5 million cases in 2015 (around 5% of the population) to 27 million cases (around 8% of the population) by 2030.[2,3] NASH progresses to fibrosis and cirrhosis in approximately 20% of patients, and 45% of patients with cirrhosis will progress to decompensated cirrhosis within 10 years.[4]

There are currently no approved disease-modifying pharmacological interventions for NASH; lifestyle modification continues to be the recommended strategy for disease management.[5] Fibrosis progression is a strong predictor of mortality and liver-related morbidity in patients with NASH, and improving fibrosis is an important goal of treatment.[6] Notably, relative reductions in hepatic fat of at least 30% correlate with histological improvements and reduced fibrosis progression.[7,8]

FGF21 is an endogenous metabolic hormone secreted by the liver, and is a key regulator of energy expenditure and glucose and lipid metabolism via activation of FGF receptors (FGFRs) in metabolically active organs.[9]

Research in context

Evidence before this study

We searched PubMed on Nov 22, 2021, for randomised controlled trials of therapies based on FGF21 analogues for the treatment of non-alcoholic steatohepatitis (NASH), using the following search string: "(non-alcoholic fatty liver disease [MeSH] OR non-alcoholic fatty liver disease OR NAFLD OR steatohepatitis OR NASH OR fatty liver) AND (Receptors, Fibroblast Growth Factor[MeSH] OR fibroblast growth factor 21 OR FGF21 OR FGF-21)". Two randomised, double-blind, placebo-controlled, phase 2a trials were identified that assessed the effects of 12–16 weeks of treatment with pegbelfermin (polyethylene glycol-conjugated [PEGylated] recombinant human FGF21) or efruxifermin (fusion of human IgG1 Fc domain with modified human FGF21), given at least weekly via subcutaneous injection to patients with biopsy-confirmed NASH. Each study reported significant reductions with the study drug versus placebo in hepatic fat fraction, liver aminotransferases (alanine aminotransferase and aspartate aminotransferase), and lipid metabolism (triglycerides, LDL and HDL cholesterol, and adiponectin). Significant reductions in N-terminal propeptide of type III collagen (PRO-C3; a marker of fibrosis) were also reported in both studies. The most common adverse events occurring more frequently with pegbelfermin or efruxifermin than with placebo were gastrointestinal in nature. Absolute reductions in hepatic fat fraction reported in the efruxifermin study (–12·3 to –14·1% with efruxifermin *vs* 0·3% with placebo) were higher than those reported in the pegbelfermin study (–6·8% to –5·2% with pegbelfermin *vs* –1·3% with placebo), but gastrointestinal adverse events appeared to be substantially more frequent with efruxifermin than with pegbelfermin.

Added value of this study

In this randomised, double-blind, placebo-controlled, phase 1b/2a study, multiple ascending doses of the glycoPEGylated FGF21 analogue pegozafermin (BIO89-100) were administered once weekly or once every 2 weeks to participants with non-alcoholic fatty liver disease (NAFLD) at high risk of NASH (referred to in this study as phenotypic NASH) or with biopsy-confirmed NASH for 12 weeks. The most common treatment-related adverse event was a mild increase in appetite, which was not associated with bodyweight gain. No other gastrointestinal-related adverse events were reported at a higher frequency in the pegozafermin groups than in the pooled placebo group. Significant, absolute reductions in hepatic fat fraction were observed at week 13 with pegozafermin once weekly and once every 2 weeks dosing, compared with an increase in the placebo group. The majority of participants had at least a 30% reduction in hepatic fat fraction, a threshold shown to correlate with histological improvements and reduced fibrosis progression in previous studies. Consistent with previous randomised controlled trials of FGF21 analogues, improvements in liver aminotransferases, the fibrosis marker PRO-C3, and lipid metabolism were observed with pegozafermin compared with placebo. These data indicate that pegozafermin could combine the promising efficacy of an FGF21 analogue with the potential added benefits of a milder adverse event profile and the possibility of less frequent dosing (ie, once every 2 weeks) for the treatment of NASH.

Implications of all the available evidence

The results of this trial further support the therapeutic potential of FGF21 analogues in patients with NASH, a disease with a high unmet medical need. The beneficial effects of these molecules on liver-related biomarkers, combined with attenuation of metabolic perturbations that underlie NASH pathology and risk factors for cardiovascular disease (a leading cause of death in these patients), are promising. The efficacy and safety of pegozafermin 15 mg once weekly, 30 mg once weekly, and 44 mg once every 2 weeks are currently being assessed in patients with NASH (NAFLD Activity Score ≥4) and fibrosis (stage 2 or 3) in the ongoing phase 2 ENLIVEN study (ClinicalTrials.gov, NCT04929483).

Pharmacological administration of FGF21 has been shown to have beneficial effects in patients with NASH. These benefits include increased hepatic insulin sensitivity, stimulation of fatty acid oxidation, inhibition of de novo lipogenesis, and decreased delivery of triglyceride-enriched VLDL via downregulation of VLDL receptor expression in hepatocytes.[10] However, native FGF21 has a short half-life (around 2 h), restricting its therapeutic potential.

Pegozafermin (BIO89-100) is a glycoPEGylated FGF21 analogue with an extended in vivo half-life that has an N-terminal methionine residue, two point mutations, and a single 20 kDa linear polyethylene glycol (PEG) covalently attached via a glycosyl moiety.[11] A first-in-human, phase 1, single-ascending-dose study in healthy volunteers showed that pegozafermin administered subcutaneously had a half-life of 55–100 h, supporting investigation of dosing both once per week and once every 2 weeks.[12] Significant beneficial changes in triglycerides, LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and adiponectin concentrations were also observed in this study.[12]

We aimed to evaluate the safety, tolerability, and pharmacokinetic and pharmacodynamic effects of multiple ascending doses of pegozafermin in participants with biopsy-confirmed NASH or with NAFLD who are at high risk of NASH (referred to in this study as phenotypic NASH).

Methods

Study design and participants

This randomised, double-blind, placebo-controlled, phase 1b/2a, multiple-ascending-dose study was conducted at 12 clinical sites (specialist centres and clinics that enrol participants in clinical trials) in the USA. The study protocol and amendments (appendix pp 6–8) were approved by the institutional review board or independent ethics committee for each site. All participants provided written informed consent.

Adults aged 21–75 years with an MRI proton-density fat fraction (MRI-PDFF) of 10% or greater and a BMI of at least 25 kg/m² were enrolled. Participants were required to have either biopsy-confirmed NASH with NASH Clinical Research Network (CRN) fibrosis stage 1, 2, or 3 based on a biopsy done in the past 24 months before screening, or phenotypic NASH if a biopsy sample was not available. Phenotypic NASH was defined as obesity (BMI >30 kg/m²) with either type 2 diabetes (fasting plasma glucose ≥126 mg/dL, 2 h plasma glucose ≥200 mg/dL in a 75 g oral glucose tolerance test, or glycated haemoglobin [HbA_{1c}] ≥6·5% [48 mmol/mol]) or evidence of liver injury (increased alanine aminotransferase [ALT] 40–200 U/L in men or 30–200 U/L in women, or FibroScan [Echosens, Waltham, MA, USA] vibration-controlled transient elastography score ≥7 kPa, or both). Individuals were excluded if they had liver disease other than NASH, evidence of cirrhosis, cardiovascular or cerebrovascular disease, or any illness that, in the opinion of the investigator, might confound the results of the study or pose an additional risk to the patient. Individuals with any clinically significant abnormality at screening in laboratory measurements, electrocardiogram (ECG), or vital signs were also excluded. Full exclusion criteria are provided in the appendix (pp 9–13).

Randomisation and masking

The principal investigators at each study site enrolled participants. Eligible individuals were centrally randomised, with participants assigned to one of six dose cohorts based on order of enrolment, and then randomly assigned within that cohort to active treatment or placebo (with allocation concealment) by use of an interactive web response system (developed, deployed, and supported by ProSciento, Chula Vista, CA, USA). The dummy participant randomisation and inventory schedules were tested in the interactive web response system to ensure the system performed according to protocol requirements. The final participant randomisation and inventory schedules were then imported into the interactive web response system by an unmasked statistician. Periodic review of the randomisation was done by the unmasked statistician throughout the study.

Participants, principal investigators, other study personnel, and the study sponsor were masked to treatment assignments throughout the study. Pegozafermin and placebo were prepared in syringes at each study site by an unmasked pharmacist. Unmasked pharmacists were not involved in any other study-related procedures. Syringes containing pegozafermin or placebo were identical, and the study drug was administered by masked site staff.

Two dosing regimens were evaluated: once weekly (3 mg [cohort 1], 9 mg [cohort 2], 18 mg [cohort 3], and 27 mg [cohort 4]) and once every 2 weeks (18 mg [cohort 5] and 36 mg [cohort 6]). Within each cohort, participants were randomly assigned to receive pegozafermin or placebo and treated for 12 weeks, with the first dose administered on day 1 and the last dose administered on day 85 (13 doses for the once weekly cohorts and seven doses for the once every 2 weeks cohorts; randomisation ratios and block sizes are reported in the appendix p 13). A safety monitoring committee was set up to review participants' safety and for dose escalation decisions. The safety monitoring committee was composed of the sponsor's medical monitor (MM), the clinical research organisation medical monitor (LM), and at least one principal investigator (BBF). There were two planned safety monitoring committee meetings for dose escalation decisions, and additional post-hoc meetings could be held as needed. A blinded safety review was done by the safety monitoring committee after participants in cohort 1 completed the day 36 visit. If no safety concerns were identified, randomisation of additional participants into

See Online for appendix

cohorts 2 and 5 was initiated. If no safety concerns were identified after at least eight participants from both cohort 2 and cohort 5 had completed the day 36 visit, including at least one participant receiving placebo in each cohort, randomisation of additional participants into cohorts 3, 4, and 6 was initiated.

Procedures

Participants were treated once weekly (in cohorts 1, 2, 3, and 4) or once every 2 weeks (in cohorts 5 and 6) with one or two subcutaneous injections of pegozafermin or placebo in the abdomen starting on day 1 and continuing through to day 85. Pegozafermin or placebo was administered by qualified study personnel. Allowed reasons for study discontinuation, withdrawal, or interruption are provided in the appendix (p 13).

Adverse events were continuously monitored throughout the study (appendix pp 15–22) and were coded with the Medical Dictionary for Regulatory Activities (version 23.0). The safety monitoring committee reviewed blinded safety data (adverse events, clinical laboratory measurements, vital signs, and ECGs).

Blood sampling for pharmacokinetic analyses was initiated on day 1 (the first dosing day), as well as on day 29 (the fifth dosing day for cohorts 1–4; the third dosing day for cohorts 5–6) when steady-state serum pegozafermin concentrations were achieved. Samples were taken before (days 1 and 29), and after dosing at 6 h, 12 h, 24 h (days 2 and 30), 48 h (days 3 and 31), 72 h (days 4 and 32), 96 h (days 5 and 33) and 168 h (days 8 and 36). For cohorts with dosing once every 2 weeks, an additional pharmacokinetic blood sample was taken 336 h after the day 43 dose (rather than the day 29 dose), but was incorporated into the day 29 steady-state pharmacokinetic analysis as the trough value for pegozafermin exposure.

Pharmacodynamic assessments (eg, MRI-PDFF and hepatic and metabolic biomarkers), laboratory tests, 12-lead ECG, and vital signs were assessed as per schedule (appendix pp 15–22).

Outcomes

The primary endpoints of the study were the safety and tolerability of pegozafermin, assessed by the frequency and severity of adverse events and serious adverse events and the number of discontinuations due to adverse events and treatment-related adverse events, and the pharmacokinetics of pegozafermin, determined by the maximal observed serum concentrations (C_{max}) within a dosing interval, area under the serum drug concentration–time curve from time zero to time of last measurable concentration within a dosing interval (AUC_{last}), time to achieve C_{max} (t_{max}), terminal elimination half-life ($t_{½}$), and accumulation ratios (AUC_{last} on day 29 [steady-state]/AUC_{last} on day 1).

Absolute and percentage changes from baseline in hepatic fat fraction (MRI-PDFF) at week 13 were key secondary endpoints. Additional secondary endpoints included absolute and percentage changes from baseline at week 13 in bodyweight, triglycerides, HDL-C, non-HDL-C, LDL-C, HbA_{1c}, homoeostatic model assessment of insulin resistance (HOMA-IR), adipose insulin resistance, liver function tests (ALT and aspartate aminotransferase [AST]), adiponectin, and N-terminal propeptide of type III collagen (PRO-C3). The immunogenicity of pegozafermin, measured by the incidence and characteristics of anti-drug antibodies (ADAs) after dosing (titre and binding specificity to the PEG or FGF21 moiety of pegozafermin, and neutralising immunogenicity), as well as the potential effects of ADAs on serum pegozafermin concentrations and safety, were also assessed as secondary endpoints. Additionally, absolute and percentage changes from baseline in hepatic volume (assessed by MRI) were key exploratory endpoints.

Other safety endpoints were incidences of, and clinically significant shifts in, vital signs, physical examination findings, ECG data, and clinical laboratory measurements, including complete blood count, biochemistry, cortisol, and urinalysis.

Statistical analysis

No formal sample size calculation was done for the primary endpoints as the number of participants (n=81) was considered adequate to achieve the safety, tolerability, and pharmacokinetic objectives of this phase 1b/2a study. In relation to changes in hepatic fat fraction (a key secondary endpoint), a power calculation showed that, compared with a pooled placebo group of 19 participants, nine participants in a dose cohort would provide around 89% power, 12 participants would provide around 93% power, and 14 participants would provide around 95% power, to detect differences between treatment groups of 30% in terms of the mean relative percentage change in MRI-PDFF from baseline, assuming an SD of 25% for this endpoint in each group. These calculations were based on a two-sample *t*-test with a one-sided 5% (two-sided 10%) type I error probability.

Statistical analysis was done with SAS (version 9.4 or higher). Six population analysis sets were defined (appendix p 23). Placebo groups from each cohort were pooled for analysis. Summary descriptive statistics were used to present demographics and baseline characteristics, safety endpoints, and pharmacokinetic and pharmacodynamic parameters. A mixed-model repeated measures analysis was used to analyse absolute changes from baseline or percentage changes from baseline in pharmacodynamic endpoints. The mixed-model repeated measures analysis included baseline as a covariate, with treatment group, visit, and the interaction between treatment group and visit as factors. The analyses were implemented with SAS PROC MIXED and the primary analysis was testing the interaction term. The covariance was unstructured. If the model failed to converge, other structures, such as compound symmetry, were considered. Least-squares means (LSMs) and LSM

differences were presented by visit with corresponding standard error, p values, and two-sided Wald interval 90% CIs and 95% CIs (data reported here relate to 95% CIs, with all 90% CIs and 95% CIs provided in the appendix pp 24–41). When strong evidence existed that normality assumptions were violated, non-parametric methods were considered, such as the Wilcoxon rank sum test. Response rates were calculated with Miettinen–Nurminen 95% CIs. Differences in response rates between placebo and pegozafermin were analysed with Fisher's exact test. No adjustments for multiplicity or data imputations for missing values were done in relation to the study outcomes.

Pre-planned subgroup analyses included changes from baseline in pharmacodynamic variables in patients with biopsy-confirmed NASH versus those with phenotypic NASH, changes from baseline in ALT concentrations in individuals with elevated ALT concentrations (defined by the central laboratory as >45 U/L) at baseline, and changes from baseline in triglycerides in individuals with elevated triglycerides (≥200 mg/dL) at baseline. Subgroup analyses were planned to be descriptive in nature, with statistical analyses (as described above) applied post hoc. Correlation of changes in ALT from baseline with changes in hepatic fat fraction from baseline was also done as a post hoc analysis by use of pairwise Pearson correlation.

The incidence and numbers of adverse events were summarised by system organ class and preferred term, and by treatment group and pooled pegozafermin or placebo groups, by use of descriptive statistics.

Data handling procedures, including data management activities such as case report form and data collection, data review, data reconciliation, and database lock were administered by IBM Clinical Development (Morrisville, NC, USA) with an electronic data capture system.

This trial is registered with ClinicalTrials.gov (NCT04048135) and is now completed.

Role of the funding source

This study was funded by 89bio, which had a role in study concept and design, data collection, data analysis, data interpretation, and writing of the clinical study report. All authors (some of whom are 89bio employees) had access to the study data, participated in developing or reviewing the manuscript, and provided final approval to submit the manuscript for publication.

Results

Between July 29, 2019, and Aug 3, 2020, 275 individuals were screened; 81 met all eligibility criteria and were randomly assigned to study treatment (62 to pegozafermin and 19 to placebo; figure 1). Of the 62 participants randomly assigned to pegozafermin, six were assigned to 3 mg once weekly, 12 to 9 mg once weekly, 11 to 18 mg once weekly, ten to 27 mg once weekly, 14 to 18 mg once every 2 weeks, and nine to 36 mg once every 2 weeks. One participant assigned to placebo in the randomised analysis set inadvertently received a single dose of 3 mg pegozafermin once weekly. The safety analysis set thus included 63 participants in the pegozafermin group and 18 in the pooled placebo group. Ten (12%) participants prematurely discontinued; reasons included adverse events (n=2), non-adherence to the study protocol (n=1), withdrawal of consent (n=6), and other reasons (n=1; figure 1). Study interruption due to COVID-19 occurred in 11 participants (two receiving placebo [one after the end of treatment] and nine receiving pegozafermin [four after the end of treatment]; duration 6–21 days).

Overall, baseline characteristics were similar among the pooled placebo and pegozafermin cohorts (table 1). Nearly all participants (77 [95%] of 81) met at least one of five criteria associated with a high risk of having NASH; 52 (64%) of 81 met at least two of these criteria (table 1). Baseline characteristics were also similar between participants with biopsy-confirmed NASH (n=15) and those with phenotypic NASH (n=66), except for type 2 diabetes, which was less prevalent in the biopsy-confirmed NASH subpopulation than in the phenotypic NASH subpopulation (four [27%] of 15 *vs* 33 [50%] of 66), and the proportion of male participants, which was lower in the biopsy-confirmed NASH subpopulation than in the phenotypic NASH subpopulation (three [20%] of 15 *vs* 28 [42%] of 66; appendix p 43).

Overall, treatment-emergent adverse events occurred in 40 (63%) of 63 participants who received pegozafermin, and in eight (44%) of 18 participants who received placebo (table 2), with most (27 [68%] of 40) being mild in severity. Two serious, non-drug-related treatment-emergent adverse events due to COVID-19 occurred in the pegozafermin group; neither led to treatment discontinuation or study withdrawal. Treatment discontinuations due to adverse events occurred in two participants: one in the 27 mg once weekly cohort who had a grade 2 skin rash deemed as possibly related to treatment; and one (with type 2 diabetes) in the 18 mg once every 2 weeks cohort who had grade 3 acute hyperglycaemia, grade 1 chest pain, and grade 1 blurred vision, all assessed as unrelated to treatment. The most common adverse events (pooled pegozafermin *vs* pooled placebo) were increased appetite (ten [16%] of 63 *vs* none), diarrhoea (eight [13%] of 63 *vs* four [22%] of 18), headache (seven [11%] of 63 *vs* one [6%] of 18), and nausea (five [8%] of 63 *vs* three [17%] of 18); of these, increased appetite and headache occurred more frequently with pegozafermin than with placebo. Increased appetite was not associated with bodyweight gain. Treatment-related treatment-emergent adverse events were reported in 24 (38%) of 63 participants in the pooled pegozafermin group and five (28%) of 18 participants in the pooled placebo group. The most common treatment-related adverse events (pooled pegozafermin *vs* pooled placebo) were increased appetite (ten [16%] of 63 *vs* none), diarrhoea (six [10%] of 63 *vs* two [11%] of 18), and headache (four [6%] of 63 *vs* one [6%] of 18). No deaths were reported.

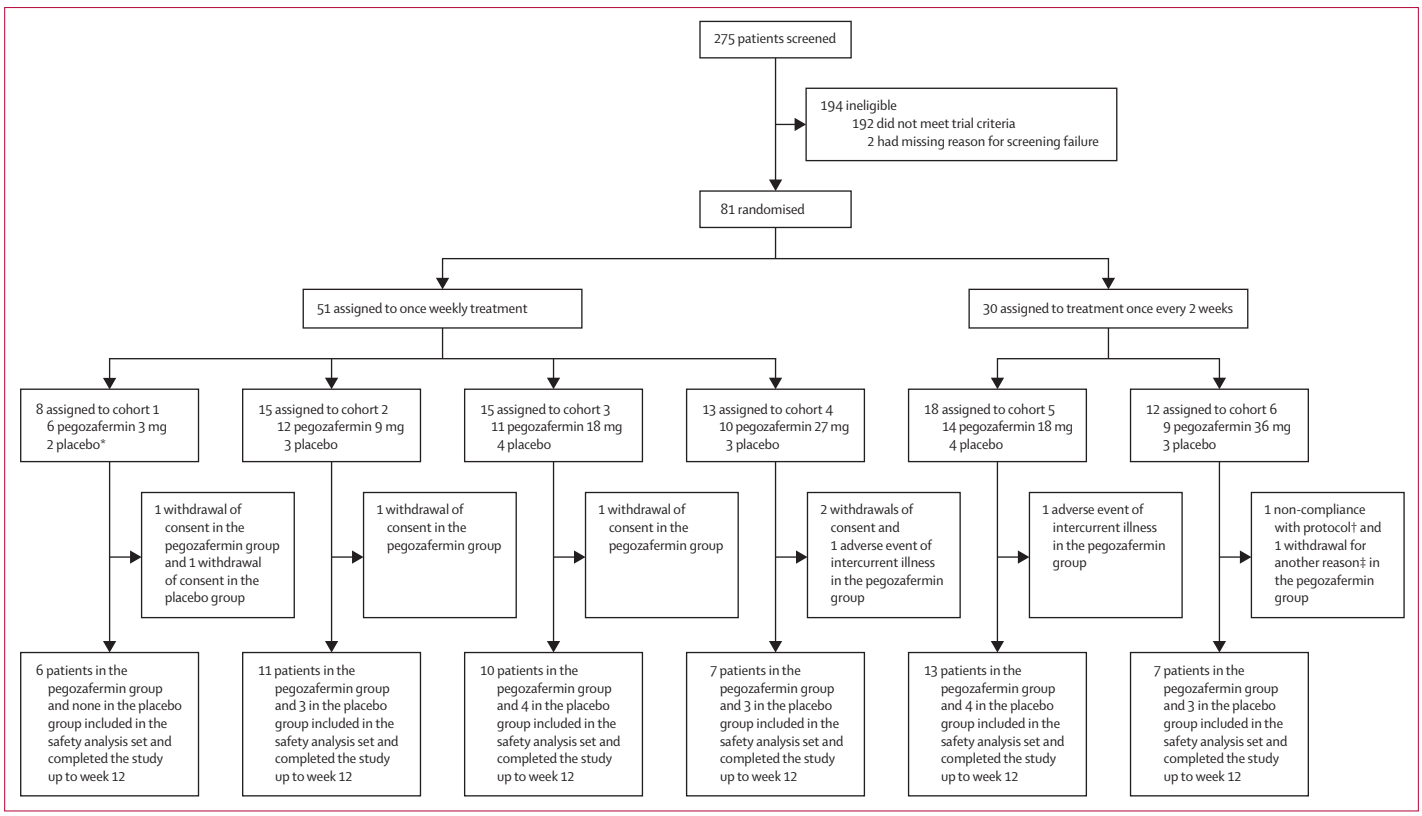

Figure 1: **Trial profile**
Dose escalation occurred at two points in the study: after cohort 1 completed the day 36 visit, the safety monitoring committee decided that further participants could be randomly assigned into cohorts 2 and 5; and after at least eight participants from both cohorts 2 and 5, including at least one participant receiving placebo in each cohort, completed the day 36 visit, the safety monitoring committee decided that further participants could be randomly assigned into cohorts 3, 4, and 6. Reasons for withdrawal were as follows: patient switched to another clinical trial (n=1 [cohort 2, pegozafermin 9 mg once weekly]); family emergency (n=2 [cohort 1, pegozafermin 3 mg once weekly; cohort 3, pegozafermin 18 mg once weekly); safety concerns (n=1 [cohort 1, placebo]); and COVID-19 related (n=2 [both in cohort 4, pegozafermin 27 mg once weekly]). *One patient randomly assigned to the placebo group in cohort 1 inadvertently received a single dose of 3 mg pegozafermin. The safety analysis set therefore comprised 63 patients in the pegozafermin group (seven in cohort 1) and 18 in the placebo group (one in cohort 1). †Participant did not adhere to study visits due to work-related travel and the decision was made by the investigator to discontinue the participant after more than 6 weeks had elapsed. ‡Incorrect discontinuation of one patient due to a COVID-19-related miscommunication with the study site.

Gastrointestinal adverse events, including diarrhoea, nausea, and abdominal pain or discomfort, occurred at similar frequencies between the pooled pegozafermin group (19 [30%] of 63) and the pooled placebo group (six [33%] of 18), with no notable differences observed among dose groups. Mild transient and self-limiting injection-site events (erythema, pain, pruritus, or reaction) were reported in four (6%) of 63 participants in the pooled pegozafermin group and none in the pooled placebo group.

Overall, ADAs were detected in 41 (65%) of 63 participants treated with pegozafermin (range across cohorts: 14–79%) at any visit. 39 (64%) of 61 participants in the pegozafermin group had treatment-induced ADAs; two participants in the pooled pegozafermin group were ADA positive at baseline. Specificity was mostly to the FGF21 domain (40 [63%] of 63 participants). Specificity to PEG was present in three (5%) of 63 pegozafermin-treated participants. The emergence of ADAs appeared to be dose related, with doses higher than 3 mg once weekly eliciting higher titres, and the number of ADA-positive participants and ADA titre increasing with duration of treatment. No differences in ADA responses were observed between the once weekly and once every 2 weeks cohorts. There was no evidence to suggest that the pharmacokinetic, pharmacodynamic, or safety profiles of pegozafermin were altered in participants with ADAs (data not shown). No neutralising antibodies were observed.

No clinically significant findings were identified on the basis of laboratory measurements, vital signs, ECG, or physical examination; specifically, no hypersensitivity reactions or tremors were reported, and no clinically relevant changes in blood pressure or heart rate were observed. No clinically significant findings on bone biomarkers (C-terminal telopeptide, procollagen type 1 N-terminal propeptide, osteocalcin, and bone-specific alkaline phosphatase) or 24 h urine cortisol assessment were observed.

At steady state on day 29, the terminal phases of the concentration–time profiles were generally parallel on semi-logarithmic plots (appendix p 3), suggesting dose-proportional pharmacokinetics, with a median $t_{½}$ of

	Placebo (n=19)	Pegozafermin (n=62)						
		3 mg once weekly (n=6)	9 mg once weekly (n=12)	18 mg once weekly (n=11)	27 mg once weekly (n=10)	18 mg once every 2 weeks (n=14)	36 mg once every 2 weeks (n=9)	Pooled (n=62)
Age, years								
Mean (SD)	52·62 (8·99)	56·13 (8·23)	49·50 (11·45)	51·47 (13·39)	51·96 (9·83)	51·22 (8·14)	52·46 (8·73)	51·71 (10·00)
Median (IQR)	56·4 (44·9–59·6)	56·7 (48·4–61·8)	51·8 (45·2–57·8)	55·1 (42·4–61·3)	51·0 (44·2–61·3)	51·8 (46·0–56·7)	53·1 (46·8–58·1)	53·5 (45·8–58·7)
Sex								
Female	12 (63%)	5 (83%)	6 (50%)	8 (73%)	8 (80%)	10 (71%)	1 (11%)	38 (61%)
Male	7 (37%)	1 (17%)	6 (50%)	3 (27%)	2 (20%)	4 (29%)	8 (89%)	24 (39%)
Race								
Black or African American	1 (5%)	1 (17%)	0	2 (18%)	0	1 (7%)	0	4 (6%)
Native Hawaiian or Pacific Islander	1 (5%)	0	0	0	0	0	0	0
White	17 (89%)	5 (83%)	11 (92%)	9 (82%)	10 (100%)	13 (93%)	9 (100%)	57 (92%)
Other	0	0	1 (8%)	0	0	0	0	1 (2%)
Ethnicity								
Hispanic or Latino	16 (84%)	6 (100%)	11 (92%)	9 (82%)	10 (100%)	13 (93%)	9 (100%)	58 (94%)
Not Hispanic or Latino	3 (16%)	0	1 (8%)	2 (18%)	0	1 (7%)	0	4 (6%)
Bodyweight, kg								
Mean (SD)	93·64 (15·26)	87·93 (23·40)	87·18 (17·33)	87·05 (17·14)	94·02 (11·00)	101·48 (18·75)	101·06 (19·80)	93·58 (18·24)
Median (IQR)	93·0 (78·0–101·2)	81·5 (76·5–89·0)	90·7 (74·5–100·0)	86·9 (77·5–92·1)	92·9 (84·0–105·4)	102·3 (88·9–119·1)	95·8 (81·4–113·8)	90·4 (81·0–105·4)
BMI, kg/m²								
Mean (SD)	33·80 (2·79)	34·25 (5·28)	32·73 (5·26)	32·77 (5·14)	36·82 (4·84)	37·01 (5·30)	34·79 (4·73)	34·81 (5·23)
Median (IQR)	33·6 (31·2–35·8)	32·6 (30·9–34·2)	31·8 (29·4–36·7)	31·3 (29·6–34·8)	37·1 (31·8–40·3)	37·7 (33·3–41·3)	34·1 (31·5–37·4)	34·1 (31·2–38·2)
History of type 2 diabetes								
No	7 (37%)	1 (17%)	8 (67%)	4 (36%)	6 (60%)	11 (79%)	7 (78%)	37 (60%)
Yes	12 (63%)	5 (83%)	4 (33%)	7 (64%)	4 (40%)	3 (21%)	2 (22%)	25 (40%)
ALT, U/L								
Mean (SD)	38·8 (21·8)	45·0 (26·8)	32·8 (16·7)	38·4 (25·3)	53·3 (46·8)	39·1 (17·4)	50·4 (27·3)	42·3 (27·4)
Median (IQR)	30·0 (22·0–48·0)	40·0 (26·0–51·0)	29·0 (22·0–34·5)	27·0 (17·0–60·0)	39·5 (23·0–61·0)	36·5 (27·0–46·0)	51·0 (28·0–66·0)	34·0 (23·0–54·0)
AST, U/L								
Mean (SD)	29·0 (16·7)	34·5 (29·2)	22·8 (7·2)	30·9 (19·3)	39·0 (25·6)	28·8 (11·3)	38·1 (36·5)	31·5 (21·7)
Median (IQR)	23·0 (17·0–35·0)	24·0 (18·0–39·0)	22·5 (16·5–26·0)	23·0 (17·0–41·0)	32·5 (25·0–39·0)	29·0 (21·0–32·0)	24·0 (21·0–32·0)	25·0 (21·0–33·0)
MRI-PDFF								
Mean (SD)	21·75% (9·01)	22·43% (8·47)	21·43% (6·30)	19·30% (6·29)	22·01% (9·19)	21·57% (8·93)	20·94% (9·54)	21·20% (7·87)
Median (IQR)	21·6% (13·3–28·7)	20·9% (14·7–27·6)	20·3% (18·7–24·5)	18·4% (12·6–26·9)	20·2% (13·9–23·3)	20·0% (13·7–28·9)	16·7% (14·7–25·2)	19·9% (14·5–26·9)
≥1/5 high-risk NAFLD criteria*	19 (100%)	6 (100%)	11 (92%)	9 (82%)	10 (100%)	13 (93%)	9 (100%)	58 (94%)
≥2/5 high-risk NAFLD criteria*	11 (58%)	5 (83%)	6 (50%)	4 (36%)	9 (90%)	11 (79%)	6 (67%)	41 (66%)
Biopsy-confirmed NASH subgroup	4 (21%)	0	0	4 (36%)	0	7 (50%)	0	11 (18%)

Data are n (%), mean (SD), or median (IQR). ALT=alanine aminotransferase. AST=aspartate aminotransferase. PDFF=proton density fat fraction. NAFLD=non-alcoholic fatty liver disease. NASH=non-alcoholic steatohepatitis. PRO-C3=N-terminal propeptide of type III collagen. ULN=upper limit of normal. *Baseline ALT >1×ULN; vibration-controlled transient elastography ≥7·0 kPa; enhanced liver fibrosis score ≥7·7; PRO-C3 ≥14·71 ng/mL; and fibrosis-4 index score >1·3.

Table 1: **Participant demographics and baseline characteristics in the randomised population**

approximately 46–68 h across cohorts. Exposure (AUC_{last}) was also generally dose-proportional across cohorts, with dose-normalised AUC_{last} fluctuating over the examined dose range with no discernible dose-related pattern (table 3). Median C_{max} values ranged from 103 ng/mL to 1674 ng/mL, and t_{max} was reached 48–72 h after dosing across cohorts. Median accumulation ratios ranged from 1·0 to 1·4 for once weekly regimens and from 1·0 to 1·1 for once every 2 weeks regimens.

At week 13, the hepatic fat fraction was significantly reduced from baseline for all evaluated pegozafermin doses compared with pooled placebo (figure 2A; appendix p 4), with the greatest effect observed in the 27 mg once

	Placebo (n=18)	Pegozafermin (n=63)						
		3 mg once weekly (n=7)	9 mg once weekly (n=12)	18 mg once weekly (n=11)	27 mg once weekly (n=10)	18 mg once every 2 weeks (n=14)	36 mg once every 2 weeks (n=9)	Pooled (n=63)
Treatment-emergent adverse events								
Participants with any treatment-emergent adverse event	8 (44%)	6 (86%)	4 (33%)	7 (64%)	7 (70%)	8 (57%)	8 (89%)	40 (63%)
Serious treatment-emergent adverse events	0	0	0	0	0	1 (7%)†	1 (11%)†	2 (3%)†
Participants with any treatment-related treatment-emergent adverse event	5 (28%)	4 (57%)	3 (25%)	3 (27%)	7 (70%)	5 (36%)	2 (22%)	24 (38%)
Participants who discontinued owing to treatment-emergent adverse event	0	0	0	0	1 (10%)	1 (7%)	0	2 (3%)
Treatment-emergent adverse events occurring in ≥10% of participants								
Increased appetite	0	4 (57%)	2 (17%)	0	2 (20%)	2 (14%)	0	10 (16%)
Diarrhoea	4 (22%)	1 (14%)	2 (17%)	0	2 (20%)	1 (7%)	2 (22%)	8 (13%)
Headache	1 (6%)	1 (14%)	0	0	2 (20%)	2 (14%)	2 (22%)	7 (11%)
Nausea	3 (17%)	1 (14%)	0	1 (9%)	0	3 (21%)	0	5 (8%)

Data are n (%). No deaths were reported. *One participant assigned to placebo in the randomised analysis set inadvertently received a single dose of 3 mg once weekly pegozafermin. The safety analysis set thus included 63 participants in the pegozafermin group and 18 in the placebo group. †All serious treatment-emergent adverse events were hospital admissions for COVID-19 and were not related to the study drug.

Table 2: **Summary of adverse events in the safety population***

	3 mg once weekly (n=6)	9 mg once weekly (n=12)	18 mg once weekly (n=11)	27 mg once weekly (n=10)	18 mg once every 2 weeks (n=14)	36 mg once every 2 weeks (n=9)
AUC_{last}, ng×h/mL	11 879 (7350–17 778)	42 576 (24 394–70 300)	95 765 (24 822–138 904)	112 632 (57 880–231 937)	78 761 (20 520–155 994)	209 436 (153 404–287 414)
AUC_{last} per dose	3960	4731	5320	4172	4376	5818
C_{max}, ng/mL	103 (59–156)	439 (238–852)	901 (200–1281)	1166 (566–2243)	649 (160–1300)	1674 (1300–2791)
t_{max}, h	59·5 (47·3–72·0)	48·0 (18·6–58·8)	48·0 (34·7–72·1)	48·1 (46·3–63·4)	72·0 (47·5–95·2)	48·0 (45·2–71·6)
$t_{½}$, h	57·1 (54·0–75·3)	46·3 (36·1–67·7)	68·1 (50·8–114·0)	53·0 (27·9–62·2)	54·4 (45·5–70·4)	53·2 (37·4–62·2)
C_{avg}, ng/mL	70·8 (43·8–105·8)	253·4 (145·4–419·1)	572·9 (146·9–816·9)	667·0 (346·3–1257·2)	234·1 (45·2–463·2)	623·3 (457·1–1182·3)
Accumulation ratio*	1·0 (0·57–1·5)	1·2 (0·72–2·0)	1·4 (0·74–2·1)	1·2 (0·81–2·0)	1·1 (0·34–3·0)	1·0 (0·85–2·4)

Data are median (90% CI). NASH=non-alcoholic steatohepatitis. AUC_{last}=area under the serum concentration time curve from time zero to time of last measurable concentration within a dosing interval. C_{max}=maximal observed serum drug concentration. t_{max}=time to reach C_{max}. $t_{½}$=terminal elimination half-life. C_{avg}=AUC_{last} per dosing interval (calculated to ease exposure comparison between dosing regimens). *Calculated as the ratio of AUC_{last} on day 29 (steady state) to AUC_{last} on day 1 (3 mg once weekly: 11 100 [7530–16 738]; 9 mg once weekly: 39 628 [17 975–59 002]; 18 mg once weekly: 61 915 [27 852–87 895]; 27 mg once weekly: 86 641 [41 600–196 670]; 18 mg once every 2 weeks: 68 803 [35 180–131 414]; 36 mg once every 2 weeks: 232 534 [102 941–259 222]).

Table 3: **Steady-state (day 29) pharmacokinetic parameters for pegozafermin after multiple dose regimens in participants with biopsy-confirmed NASH or phenotypic NASH in the pharmacokinetics population**

weekly cohort. LSM absolute differences in hepatic fat fraction versus pooled placebo were –8·9% (95% CI –14·8 to –3·1; p=0·0032) for 3 mg once weekly, –11·5% (–16·1 to –6·9; p<0·0001) for 9 mg once weekly, –8·9% (–13·7 to –4·2; p=0·0004) for 18 mg once weekly, –14·9% (–20·1 to –9·7; p<0·0001) for 27 mg once weekly, –10·4% (–14·7 to –6·1; p<0·0001) for 18 mg once every 2 weeks, and –11·1% (–16·2 to –6·0; p<0·0001) for 36 mg once every 2 weeks. The LSM placebo-adjusted relative change in hepatic fat fraction from baseline to week 13 is shown in figure 2B; the largest change was –70·2% (95% CI –92·5 to –47·9; p<0·0001) versus pooled placebo in the 27 mg once weekly cohort (figure 2B). Hepatic volume was also significantly reduced from baseline at week 13 in most pegozafermin cohorts compared with pooled placebo (appendix p 25).

A relative reduction in hepatic fat fraction of at least 30% at week 13 was observed in the majority of patients in all

pegozafermin cohorts, versus none in the pooled placebo group (appendix p 5). In the 27 mg once weekly cohort, six (86%) of seven participants had a relative reduction in hepatic fat fraction of at least 30% at week 13, and five (71%) of seven had a relative reduction in hepatic fat fraction of at least 50%. Baseline characteristics are presented in the appendix (p 44) for participants who had a relative reduction in hepatic fat fraction of 30% or greater versus those who did not. In the 27 mg once weekly cohort, hepatic fat fraction was normalised (<5%) in three (43%) of seven participants versus none in the pooled placebo group (p=0·0152; appendix p 5). Changes in hepatic fat fraction were similar among participants with biopsy-confirmed NASH and those with phenotypic NASH treated with pegozafermin or placebo (appendix pp 45–46).

Treatment with pegozafermin significantly reduced ALT concentrations from baseline at week 13 in most of the dose cohorts (figure 3A), with the largest LSM relative change of –43·7% (absolute change –30·0 U/L) observed in the 27 mg once weekly cohort compared with –4·2% (absolute change –3·4 U/L) in the pooled placebo group (relative difference *vs* pooled placebo –39·5% [95% CI –59·9 to –19·2]; p=0·0002, and absolute difference –26·6 U/L [95% CI –39·2 to –13·9]; p<0·0001). Overall, greater ALT reductions were observed with increasing pegozafermin doses (figure 3A). Reductions in ALT from baseline at week 13 were particularly pronounced in participants with elevated ALT concentrations at baseline (LSM absolute change –34·6 U/L [n=17] for pooled pegozafermin *vs* –10·3 U/L for pooled placebo [n=5]; difference –24·3 U/L [95% CI –47·6 to –0·9]; p=0·0426; appendix p 27). A reduction in ALT concentration of 17 U/L or greater was observed in five (71%) of seven participants in the 27 mg once weekly group (p=0·0169 *vs* pooled placebo), compared with three (17%) of 18 participants in the pooled placebo group. A reduction in ALT concentration of 30% or greater was observed in five (71%) of seven participants in the 27 mg once weekly group (p=0·0069 *vs* pooled placebo), six (46%) of 13 participants in the 18 mg once every 2 weeks group (p=0·0429 *vs* pooled placebo), and five (71%) of seven participants in the 36 mg once every 2 weeks group (p=0·0069 *vs* pooled placebo), compared with two (11%) of 18 participants in the pooled placebo group. No significant differences versus pooled placebo in ALT response rates were observed for the other cohorts (appendix p 5). In pegozafermin-treated participants, reductions in hepatic fat fraction were significantly correlated with reductions in ALT concentrations (*r*=0·540; p<0·0001; appendix p 4).

The LSM relative change from baseline in AST at week 13 was –37·9% (absolute change –14·5 U/L) in the 27 mg once weekly cohort versus –4·4% (absolute change –5·2 U/L) in the pooled placebo group (relative difference –33·5% [95% CI –51·4 to –15·5]; p=0·0004 and absolute difference –9·3 U/L [95% CI –16·8 to –1·8]; p=0·0158; figure 3B). Significant reductions in AST at week 13 were also observed for the 3 mg once weekly and 18 mg once every 2 weeks dose groups compared with placebo (figure 3B; appendix p 28).

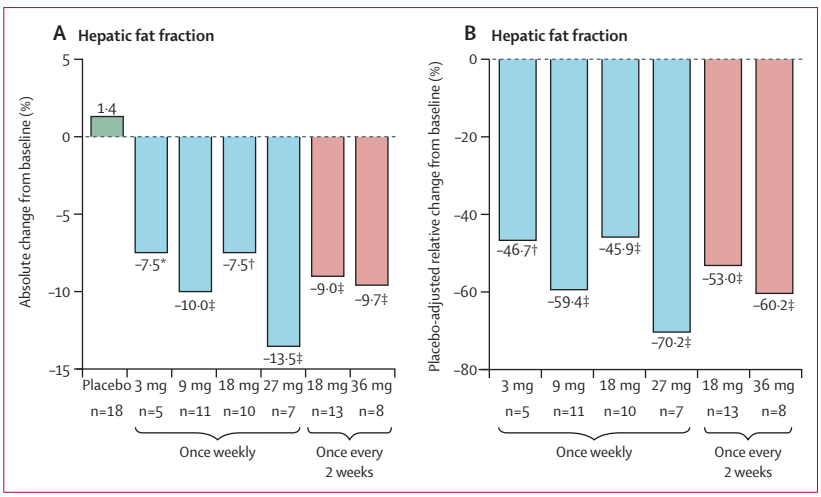

Figure 2: **Effects of pegozafermin on hepatic fat fraction (assessed by MRI-PDFF) and hepatic volume**
(A) Absolute change from baseline in hepatic fat fraction across pegozafermin dosing regimens at week 13, as assessed by MRI proton-density fat fraction (MRI-PDFF). (B) Placebo-adjusted relative change from baseline in hepatic fat fraction across pegozafermin dosing regimens at week 13, as assessed by MRI-PDFF. Data shown are least squares means. *p<0·01 versus placebo. †p<0·001 versus placebo. ‡p<0·0001 versus placebo.

A LSM relative reduction from baseline in PRO-C3 concentrations of –27·7% was observed at week 13 in the 27 mg once weekly group compared with 3·3% in the pooled placebo group (difference –30·9% [95% CI –57·4 to –4·5]; p=0·0227). Reductions in PRO-C3 concentrations were also observed in the other pegozafermin cohorts, except for the 18 mg once weekly cohort, but were not significant (figure 3C).

Reductions in ALT, AST, and PRO-C3 concentrations with pegozafermin treatment were similar in the biopsy-confirmed NASH and phenotypic NASH subpopulations (appendix pp 47–52).

At week 13, LSM reductions from baseline in triglycerides (figure 3D), LDL-C (figure 3E), and non-HDL-C (appendix pp 33–34), and increases in HDL-C (figure 3F), were observed with pegozafermin treatment compared with placebo. Triglyceride concentrations were normalised (<150 mg/dL) in eight (53%) of 15 participants in the pooled pegozafermin group (versus none in the pooled placebo group) who had high baseline triglyceride concentrations (≥200 mg/dL) at baseline.

At week 13, some improvements in insulin sensitivity, fasting plasma glucose, and HbA_{1c} were observed in cohorts that received higher (*vs* lower) doses of pegozafermin compared with those in the pooled placebo group, but none was statistically significant (appendix pp 34–38). There was a significant decrease in LSM bodyweight at week 12 in the 27 mg once weekly cohort (LSM difference *vs* placebo –2·18% [95% CI 4·2 to – 0·12]; p=0·0380). However, the placebo-adjusted LSM change in absolute bodyweight was not significant in this group. A 65·1% (95% CI 36·6 to 93·7) LSM increase in adiponectin relative to pooled placebo was observed in the 27 mg once

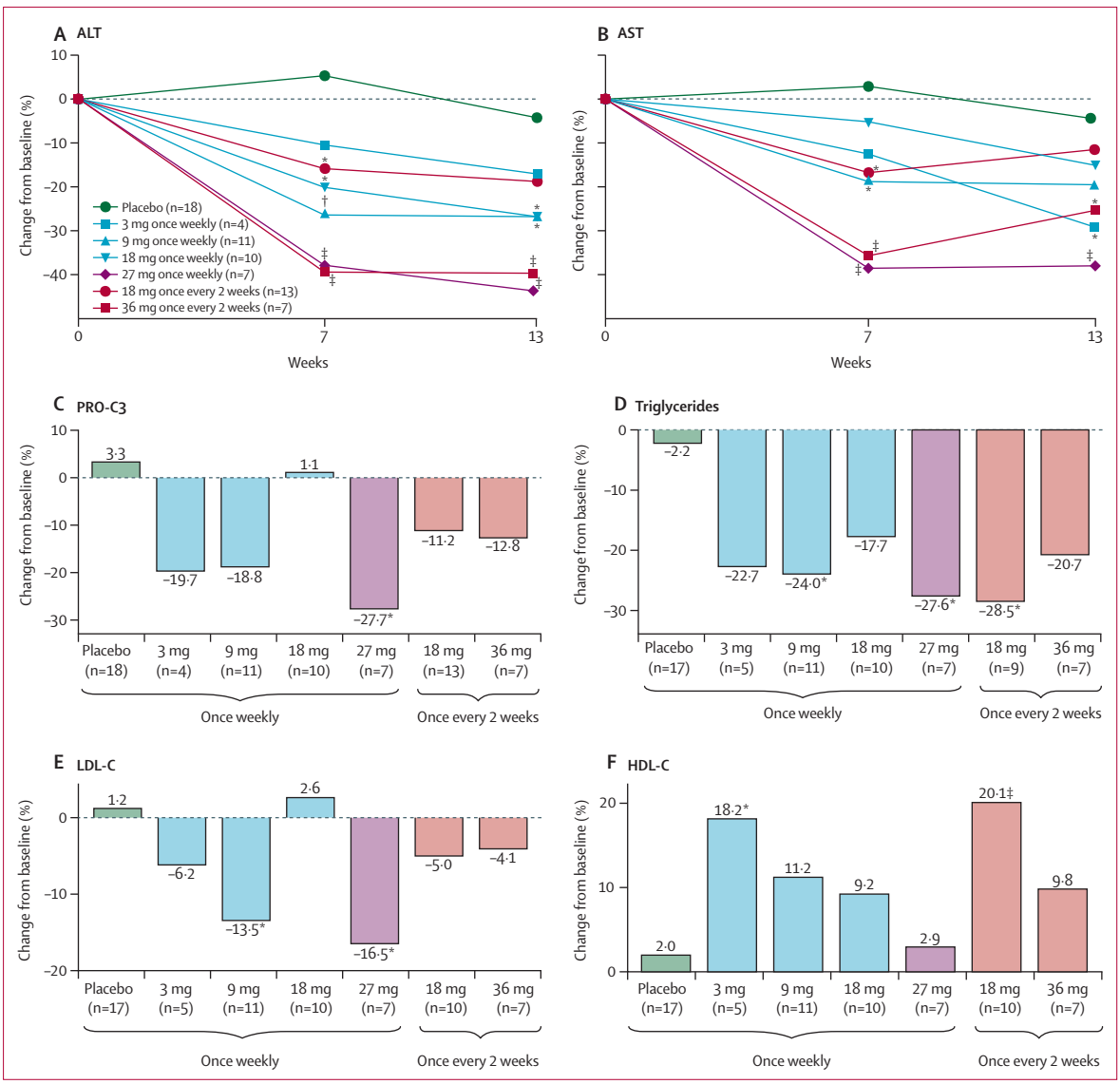

Figure 3: **Effects of pegozafermin on liver aminotransferases, PRO-C3, lipids, and metabolic markers**
(A) Percentage change from baseline in ALT across pegozafermin dose regimens. (B) Percentage change from baseline in AST across pegozafermin dose regimens. (C) Percentage change from baseline in PRO-C3 at week 13. (D) Percentage change from baseline in serum triglycerides across pegozafermin dosing regimens at week 13. (E) Percentage change from baseline in LDL-C across pegozafermin dosing regimens at week 13. (F) Percentage change from baseline in HDL-C across pegozafermin dosing regimens at week 13. ALT=alanine aminotransferase. AST=aspartate aminotransferase. HDL-C=high-density lipoprotein cholesterol. LDL-C=low-density lipoprotein cholesterol. PRO-C3=N-terminal propeptide of type III collagen. *p<0·05 versus placebo. †p<0·01 versus placebo. ‡p<0·001 versus placebo.

weekly cohort (p<0·0001; appendix pp 39–40), and significant increases of lower magnitude were observed in the other pegozafermin groups, with the exception of 36 mg once every 2 weeks group. No notable improvements from baseline were observed with pegozafermin versus placebo at week 13 with respect to changes in free fatty acid concentrations or adipose tissue insulin resistance (appendix pp 40–42).

Discussion

In this randomised, double-blind, phase 1b/2a, multiple-ascending-dose study, treatment with pegozafermin led to marked improvements in several liver-related and metabolic variables in patients with biopsy-confirmed NASH or phenotypic NASH, and was generally well tolerated. These benefits were observed across all tested doses, in once weekly and once every 2 weeks dosing cohorts, with the most prominent effects observed at the highest tested doses: 27 mg once weekly and 36 mg once every 2 weeks.

Treatment with pegozafermin for 12 weeks led to marked reductions in hepatic fat fraction as assessed by MRI-PDFF. High proportions of participants had relative reductions in hepatic fat fraction of 30% or greater and

50% or greater, which correlate with clinically relevant histological outcomes (eg, ≥2-point reduction in NAS and NASH resolution).[7,8] Hepatic volume was also significantly reduced in participants treated with pegozafermin. In participants with NASH, hepatic volume is highly correlated with MRI-derived measures of hepatic fat burden (including MRI-PDFF and total liver fat index) and with histological steatosis grade,[13] and the observed change in hepatic volume in pegozafermin-treated individuals is aligned with the marked reduction in hepatic fat fraction. It remains to be determined whether a reduction in hepatic volume will translate into additional, clinically meaningful outcomes, such as a reduction in right upper quadrant discomfort. Treatment with pegozafermin also led to significant reductions in ALT and PRO-C3 concentrations. ALT reductions of at least 17 U/L have been shown to correlate with histological improvements.[14] This ALT threshold was reached by five (71%) of seven participants in the pegozafermin 27 mg once weekly group, in whom significant reductions of PRO-C3 (a neo-epitope marker of type III collagen formation and an emerging non-invasive biomarker of fibrogenesis and fibrosis[15]) were also observed. These data suggest that pegozafermin has important benefits across multiple liver-related biomarkers that might predict a beneficial effect on clinically significant histological and other endpoints in NASH. The effect of pegozafermin on NASH histological endpoints (NAS ≥4, NASH CRN fibrosis stage 2 or 3) is currently being evaluated in a phase 2 study (ENLIVEN; NCT04929483).

NASH is commonly regarded as a hepatic manifestation of metabolic syndrome, and the term metabolic-associated fatty liver disease (MAFLD) has been suggested as a potentially more accurate term for this condition.[16] An ideal treatment for NASH would thus simultaneously address liver-related factors (eg, hepatocyte stress, immune cell infiltration, and fibrosis) and the underlying metabolic overload that drives hepatic pathology. Indeed, patients with NASH often have multiple cardiovascular risk factors, and are at high risk of cardiovascular events and cardiovascular mortality, and NAFLD or NASH itself might confer additional cardiovascular risk, particularly in patients with advanced fibrosis.[17] Importantly, cardiovascular mortality is a leading cause of death in patients with NASH.[17] In view of these considerations, it is encouraging that 12 weeks of pegozafermin treatment resulted in clinically meaningful metabolic improvements, including reductions in triglycerides, LDL-C, and non-HDL-C, and increased HDL-C, in addition to significant liver-related benefits. The concurrent reduction in hepatic fat fraction and triglycerides is noteworthy, as it has been suggested that fibrates, which are approved to treat hypertriglyceridaemia, might increase hepatic fat content and volume.[18] Notably, these benefits occurred in the absence of clinically significant safety concerns and corroborate previous findings in healthy volunteers.[12]

Improvements in additional metabolic variables (HOMA-IR, fasting plasma glucose, and HbA_{1c}) were observed at higher pegozafermin doses compared to placebo over the 12-week treatment period, but these benefits were not statistically significant. A small, significant bodyweight loss of 2·18% was observed in the 27 mg once weekly group, which was not secondary to gastrointestinal adverse events. This bodyweight loss is unlikely to have significantly contributed to the beneficial effects on serum lipids, which were also observed in other dose groups, in which bodyweight loss did not occur. The mechanism of bodyweight loss has not been investigated in this study; notably, pegozafermin has been shown to increase energy expenditure in CD-1 mice,[19] and to decrease preference for sweetness in cynomolgus monkeys.[11] In the present study, treatment with pegozafermin resulted in an increase in adiponectin, an insulin-sensitising, anti-inflammatory, anti-fibrotic, anti-atherosclerotic, and hepatoprotective factor predominantly produced by adipocytes.[20] FGF21 potently induces adiponectin gene expression in adipocytes through a peroxisome proliferator-activated receptor-γ (PPARγ)-dependent mechanism in mice, and various findings suggest that adiponectin is an important downstream mediator of FGF21, facilitating its pleiotropic effects in major peripheral organs, including the liver, via abundantly expressed adiponectin receptors.[21]

Two other FGF21 analogues, pegbelfermin (a PEGylated FGF21 analogue) and efruxifermin (an Fc-FGF21 analogue) are in clinical development for the treatment of NASH. FGF21 and its cofactor β-klotho signal via their cognate receptors FGFR1c, FGFR2c, and FGFR3, which are expressed across multiple organs, including the liver, adipose tissue, muscle, pancreas, and brain, contributing to the systemic effects observed in response to FGF21 agonism. Unlike FGF19 analogues, FGF21 does not signal via the FGFR4/β-klotho complex, the activation of which induces suppression of bile acids, leading to increased LDL-C concentrations.[22] Gastrointestinal adverse events were the most frequently reported events for pegbelfermin[23] and efruxifermin;[24] by contrast, the frequency of gastrointestinal adverse events observed with pegozafermin in the current study was similar to that of placebo. Tremors, which have been reported with efruxifermin, were not observed.

Overall, the magnitude of the anti-steatotic effect observed with FGF21 analogues is greater than that of other therapies under clinical evaluation (relative reduction of up to 70% *vs* 18–58% for other agents), such as thyroid hormone receptor β, PPAR agonists, farnesoid X receptor (FXR) agonists, FGF19, and glucagon-like peptide 1, underscoring the potential of this drug class to target intrahepatocyte fat deposition, the main driver of NASH.[23–29] Additionally, unlike FGF21 analogues, some other therapies in development for NASH might increase cardiovascular risk by increasing LDL-C (FXR agonists[25,30,31] or FGF19 analogues[32]), increasing triglyceride

concentrations (acetyl-CoA carboxylase inhibitors),[33] or promoting bodyweight gain or fluid retention, or both (PPAR agonists).[34]

The main limitations of this study were the small sample size and relatively short treatment duration. Additionally, only a subset of participants had biopsy-confirmed NASH at baseline. The mixing of histological and non-invasive inclusion criteria might have increased the heterogeneity of our study population, although most baseline characteristics, including hepatic fat fraction as assessed by MRI-PDFF, were similar in the biopsy-confirmed NASH and phenotypic NASH subpopulations, as were the observed treatment effects. Finally, where biopsy samples were available for the determination of biopsy-confirmed NASH, these were classified by local pathologists. An ongoing phase 2b study (ENLIVEN; ClinicalTrials.gov, NCT04929483), with centrally read biopsies done at baseline and after 24 weeks of treatment, followed by a blinded extension phase for a total of 48 weeks of treatment, will further evaluate the efficacy, safety, and tolerability of pegozafermin (15 mg once weekly, 30 mg once weekly, and 44 mg once every 2 weeks dosing) in participants with biopsy-confirmed NASH (NAS ≥4, NASH CRN fibrosis stage 2 or 3).

In summary, in this study, participants with biopsy-confirmed NASH and phenotypic NASH treated with pegozafermin for 12 weeks had clinically meaningful improvements in liver and metabolic parameters. These benefits were observed with pegozafermin once weekly and once every 2 weeks. Overall, the treatment was associated with a favourable safety and tolerability profile. Pegozafermin therefore has considerable potential as a therapeutic agent for the treatment of NASH and other metabolic diseases.

Contributors

RL contributed to the study concept, design, and implementation, as well as to the analysis and interpretation of the study data. EJL contributed to the study concept and implementation, as well as to the collection and interpretation of the study data. GO-L contributed to the study implementation and collection of the study data. BBF and LJ contributed to the study implementation, as well as to the collection, analysis, and interpretation of the study data. LM contributed to the study design and implementation, as well as to the collection, analysis, and interpretation of the study data. MR, C-YC, LT, RWC, HM, and MM contributed to the study concept, design, and implementation, as well as to the collection, analysis, and interpretation of the data. JPF and CLH contributed to the interpretation of the study data. All authors reviewed the manuscript for important intellectual content and approved the final version for submission, except for MR who died before the manuscript was drafted. MM and RL accessed and verified the data. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

RL reports consultant fees from Aardvark Therapeutics, Altimmune, Anylam/Regeneron, Amgen, Arrowhead Pharmaceuticals, AstraZeneca, Bristol Myers Squibb, CohBar, Eli Lilly, Galmed, Gilead, Glympse bio, Hightide, Inipharma, Intercept, Inventiva, Ionis, Janssen, Madrigal, Metacrine, NGM Biopharmaceuticals, Novartis, Novo Nordisk, Merck, Pfizer, Sagimet, Theratechnologies, 89bio, Terns Pharmaceuticals, and Viking Therapeutics; institutional research grant support from Arrowhead Pharmaceuticals, AstraZeneca, Boehringer-Ingelheim, Bristol Myers Squibb, Eli Lilly, Galectin Therapeutics, Galmed Pharmaceuticals, Gilead, Intercept, Hanmi, Intercept, Inventiva, Ionis, Janssen, Madrigal Pharmaceuticals, Merck, NGM Biopharmaceuticals, Novo Nordisk, Merck, Pfizer, Sonic Incytes, and Terns Pharmaceuticals; and is a co-founder of LipoNexus Inc. EJL reports grants or contracts from 89bio, Akero Therapeutics, Alnylam Pharmaceuticals, Amgen, AstraZeneca, Axcella Health, Boehringer Ingelheim, Bristol Myers Squibb, CytoDyn, Durect Corporation, Eli Lilly and Company, Enanta Pharmaceuticals, Galectin Therapeutics, Galmed Pharmaceuticals, Genentech, Gilead Sciences, Hanmi Pharmaceuticals, Intercept Pharmaceuticals, Janssen Pharmaceuticals, Laboratory for Advanced Medicine, Madrigal Pharmaceuticals, Merck & Co., Metacrine, NGM Biopharmaceuticals, Northsea Therapeutics, Novartis, Novo Nordisk, Pfizer, Poxel, Roche, Sagimet Biosciences, Terns Pharmaceuticals, Valeant Pharmaceuticals, Viking Therapeutics, and Zydus Pharmaceuticals; and consultant fees from Boehringer Ingelheim, Bristol Myers Squibb, Metacrine, Sagimet Biosciences, and Terns Pharmaceuticals. JPF reports grants or contracts for their institution from 89bio, Akero, AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Carmot Therapeutics, Eli Lilly, Intercept, IONIS, Janssen, Madrigal, Metacrine, Merck, NorthSea Therapeutics, Novartis, Novo Nordisk, Oramed, Pfizer, Poxel, and Sanofi; consulting fees from Akero, Altimmune, Axcella Health, Becton Dickenson, Boehringer Ingelheim, Carmot Therapeutics, Echosens, 89bio, Eli Lilly, Gilead, Intercept, Metacrine, Merck, Novo Nordisk, and Pfizer; payment or honoraria (for lectures, presentations, speakers bureaus, manuscript writing, or educational events) from Eli Lilly, Merck, Sanofi, Echosens, and Novo Nordisk; support for attending meetings or travel, or both, from Eli Lilly and Novo Nordisk; and participation in data safety monitoring boards or advisory boards for, 89bio, Carmot Therapeutics, Eli Lilly, Sanofi, Merck, Pfizer, Gilead, Intercept, and Beckton Dickenson. LJ is an employee of, and has stock or stock options in, Antaros Medical, which received grants or contracts from the study sponsor for services performed in the study. BBF was an employee of ProSciento at the time of the study, which received grants or contracts from the study sponsor for services performed in the study; received support or travel (as a principal investigator), or both, for attending the investigator meeting at the start of the study; and participated in data safety monitoring for the study. LM reports consulting fees from, and has stock or stock options in, ProSciento, which received grants or contracts from the study sponsor for services performed in the study. MR (deceased) was an employee of 89bio at the time of the study. C-YC was an employee of 89bio at the time of the study. RWC is an employee of 89bio; has stock or stock options in 89bio, Gilead, and Ascendis Pharma; and is a volunteer (unpaid) faculty committee member for the Pediatric Endocrine Society Fellow's Retreat. LT is an employee of, and has stock or stock options in, 89bio; and holds a patent for the dosing regimen of the study drug. CH, HM, and MM are employees of, and have stock or stock options in, 89bio. GOL reports no competing interests.

Data sharing

Data pertaining to the current study will not be shared.

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