Statistical Analysis Plan

A Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy, Safety and Tolerability of BIO89-100 in Subjects with Biopsy-Confirmed Nonalcoholic Steatohepatitis (NASH)

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ABBREVIATIONS

Abbreviation Term	Description
ADA	Anti-drug antibody
AE	Adverse event
AI	Artificial intelligence
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANCOVA	Analysis of covariance
AST	Aspartate transaminase
ATC	Anatomical Therapeutic Chemical
BMD	Bone mineral density
BMI	Body mass index
BUN	Blood urea nitrogen
CI	Confidence interval
CLDQ-NAFLD-NASH	Chronic Liver Disease Questionnaire - NAFLD-NASH
CMH	Cochran-Mantel-Haenszel
CN	Reported Unit
COVID-19	Coronavirus disease 2019
CRF	Case Report Form
CRN	Clinical Research Network
CSR	Clinical Study Report
cT1	Iron-corrected T1
CTCAE	Common terminology criteria for adverse events
CTX	Carboxy-terminal collagen crosslinks
DBP	Diastolic blood pressure
DILI	Drug-induced Liver Injury
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
DXA	Dual X-ray absorptiometry
ECG	Electrocardiogram
ELF	Enhanced liver fibrosis
EOS	End of study
EQ-5D-5L	Europe Quality of Life Group 5 dimension 5 level questionnaire
ET	Early Termination
FAS	Full Analysis Set
FDA	United States Food and Drug Administration
FGF21	Fibroblast growth factor 21
FIB-4	Fibrosis-4
FSH	Follicle stimulating hormone
FT4	Free thyroxine

Abbreviation Term	Description
GGT	Gamma-glutamyl transpeptidase
HbA1c	Glycated hemoglobin
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HDL-c	High density lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment for insulin resistance
ICH	International Council for Harmonisation
IGF-1	Insulin-like growth factor-1
IP	Investigational product
IRT	Interactive Response Technology
LDL-c	Low density lipoprotein cholesterol
LLOQ	Lower limit of quantitation
LS	Least square
MCH	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple imputation
MMRM	Mixed model repeated measures
MRI	Magnetic resonance imaging
MRI-PDFF	Magnetic resonance imaging-estimated proton density fat fraction
NAb	Neutralizing antibodies
NAFLD	Nonalcoholic fatty liver disease
NAS	NAFLD Activity Score
NASH	Nonalcoholic steatohepatitis
Non-HDL-c	Non-HDL cholesterol
NSC	Night-time salivary cortisol
P1NP	N-terminal propeptide of type 1 collagen
PD	Pharmacodynamic
PEG	Polyethylene glycol
PGx	Pharmacogenomics
PI	Principal Investigator
PK	Pharmacokinetic
PRO	Patient reported outcome
Pro-C3	N-terminal type III collagen propeptide
PT	Preferred Term
Q2W	Every 2 weeks
QRS	Complex in ECG representing ventricular depolarization
QTcF	Fridericia corrected QT interval in ECG

Abbreviation Term	Description
QW	Weekly
RBC	Red blood cell
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SBP	Systolic blood pressure
SC	Subcutaneous
SD	Standard deviation
SE	Standard error
SI	Standard Unit
SoA	Schedule of activities
SUSAR	Suspected unexpected serious adverse event
T2DM	Type 2 diabetes mellitus
TEAE	Treatment-emergent adverse event
TSH	Thyroid stimulating hormone
TT3	Total triiodothyronine
ULN	Upper limit of normal
ULOQ	Upper limit of quantitation
VAS	Visual analog scale
VCTE	Vibration-controlled transient elastography
WBC	White blood cell
WHO-DD	World Health Organization-Drug dictionary
WPAI-NASH	Work Productivity and Activity Impairment Questionnaire for NASH

1. INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to describe the procedures, statistical methodology, and planned analyses that will be used to analyze and report results for Protocol BIO89-100-122. Mock tables, listings, and figure shells will be provided in separate supporting documents.

This SAP complies with the International Council for Harmonisation (ICH) Harmonised Tripartite Guideline Topic E9, Statistical Principles for Clinical Trials. The current version of the SAP is based on the following study documents:

- Protocol, Version (4.0) dated 05 December 2022
- Case report form (CRF), Version 1.11 dated 24 January 2023

The document may evolve over time to reflect protocol amendments, regulatory discussions, and other important changes. However, the final SAP will be approved by the Sponsor, and placed on file before the database is locked. Any additional analyses or deviations from the final approved plan will be documented with rationale in the final clinical study report (CSR).

2. STUDY OBJECTIVES

This randomized, double-blind, placebo-controlled Phase 2b study is designed to assess the efficacy, safety, and tolerability of 3 dose regimens of BIO89-100[1](2 dose levels to be administered weekly [QW] and one dose level to be administered once every 2 weeks [Q2W]) in subjects with biopsy-confirmed nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD) activity score (NAS) ≥4, fibrosis stage F2 or F3 per NASH Clinical Research Network (CRN) system. The study will allow evaluation of the potential histological benefit of pegozafermin and the overall benefit-risk associated with administration of pegozafermin in the target population.

2.1. Main Study Objectives

2.1.1. Primary Objective

The primary objective of the Main Study is to evaluate the effect of pegozafermin on liver histology after 24 weeks of treatment.

2.1.2. Key Secondary Objective

The key secondary objective of the Main Study is to further evaluate the effect of pegozafermin on liver histology after 24 weeks of treatment.

[1] Hereafter referred to as pegozafermin.

2.1.3. Secondary Objectives

- The secondary objectives of the Main Study are:
- To evaluate the effects of pegozafermin on liver parameters
- To evaluate the metabolic effects of pegozafermin
- To characterize pegozafermin pharmacokinetics (PK) profile

2.1.4. Safety Objectives

The safety objectives of the Main Study are:

- To evaluate the safety and tolerability of pegozafermin.
- To evaluate the immunogenicity of pegozafermin.

2.1.5. Exploratory Objectives

The exploratory objectives of the Main Study are:

- To evaluate effect of pegozafermin on additional clinical parameters
- To evaluate the effects of pegozafermin on patient reported outcome (PRO)
- To evaluate molecular data (e.g., proteomics, genetics) to increase the understanding of pegozafermin biological activity, and to identify potential existing and/or emerging biomarkers.
- To explore pharmacogenomics (PGx).

2.2. Extension Study Objectives

2.2.1. Primary Objective

The primary objective of the Extension Study is to evaluate the long-term safety and tolerability of pegozafermin.

2.2.2. Secondary Objectives

The secondary objectives of the Extension Study are:

- To characterize effect of pegozafermin on liver parameters
- To characterize pegozafermin PK profile

2.2.3. Safety Objectives

The safety objectives of the Extension Study are:

- To evaluate additional safety and tolerability measures
- To evaluate the immunogenicity of pegozafermin over time

2.2.4. Exploratory Objectives

The exploratory objectives of the Extension Study are:

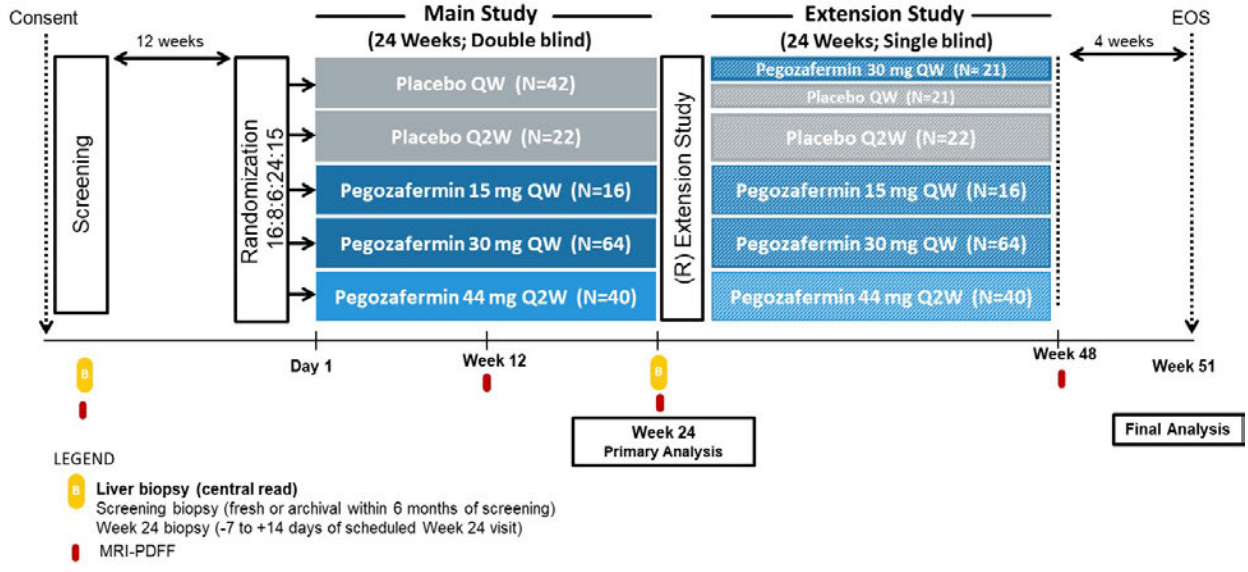
- To evaluate pegozafermin on additional metabolic parameters
- To evaluate pegozafermin on additional clinical parameters
- To evaluate the effects of pegozafermin on PRO

3. STUDY DESIGN

3.1. Study Population

Study BIO89-100-122 is a randomized, double-blind, placebo-controlled, 2-part study to evaluate efficacy, safety, tolerability, population PK and pharmacodynamic (PD) profiles and immunogenicity of pegozafermin administered subcutaneously (SC) in approximately 184 patients with biopsy-confirmed NASH (NAS ≥4, fibrosis stage F2 or F3 per NASH CRN system). The first part of the study will be double-blind, and the second part of the study will be single-blind (specifically-identified Sponsor personnel will not be blinded). Study schema is shown in Figure 1.

Figure 1: Study Schema



Abbreviations: EOS, end of study; QW, weekly; Q2W, every other week; R, At Week 24, subjects randomized to placebo QW in the Main study will be re-randomized 1:1 to pegozafermin 30 mg (n=21) or placebo QW (n=21)

Notes: The 12-week screening period may be extended with Sponsor's approval. Subjects, investigators and site staff and Sponsor will be blinded to investigational product (placebo or pegozafermin) in the Main study. During the Extension study, the Medical Monitor will remain blinded to treatment assignment. Designated members of the unblinded team are specified in the Sponsor Unblinding Plan. Subjects, investigators, and site staff will remain blinded to IP.
Subject assigned to placebo QW in the Main study will be re-randomized 1:1 to pegozafermin 30 mg QW or placebo QW at the Week 24 visits and will receive the re-randomized treatment at the Week 26 visit.
Treatment duration for Main Study includes 24 administrations in QW dose regimen and 12 administrations in Q2W dose regimen + 1 week for evaluation of endpoints (Week 24); treatment duration for Extension includes 24

administrations in QW dose regimen and 12 administrations in Q2W dose regimen + 1 week for evaluation of endpoints (Week 48).
The study includes an immunogenicity follow-up period of up to one year with follow-up visits approximately every 12 weeks after the Week 48 visit.
Abbreviations: EOS, end of study; QW, weekly; Q2W, every 2 weeks, R, randomization.

The study will include 2 parts:

- • Main Study – a 24-week, double-blind, placebo-controlled study
- • Extension Study – an additional 24-week, single-blind, placebo-controlled study.

The entire study will include a Screening period, a Treatment period (Main and Extension), and a Follow-up period. Study visits and assessments will be conducted in-clinic and/or remotely as shown in the schedule of activities (SoA) in Appendix 1.

Visits at Screening, Baseline (Day 1), Weeks 4, 12, 24, 38, and 48 should be performed in-clinic. If circumstances preclude this possibility (e.g., restrictions due to coronavirus disease 2019 [COVID-19]), the site should contact the Medical Monitor (or designee) to discuss the best way to capture appropriate data. Visits at Weeks 8, 16, and 30 may be performed remotely, with home health nursing to perform assessments (these visits may be performed in clinic if the Investigator feels it is in the best interest of the subject). The Week 26 visit may be an in-clinic or a remote (home) only visit to administer investigational product (IP), so that placebo subjects who are re-randomized to active treatment will be observed the first time they receive IP. All subjects will be followed up for at least 4 weeks after last dose of IP.

For immunogenicity assessment, subjects who complete the Week 48 visit will be asked to return to clinic to have blood samples collected approximately every 3 months for up to one year. AEs and concomitant medication will also be recorded during the immunogenicity follow-up period. Additional testing may be requested in the event of safety-related concerns. The sponsor may elect to terminate participation in the immunogenicity follow-up period based on emerging data. Subjects who terminate IP early will not return for immunogenicity follow-up, unless asked to return for additional testing(s), at the Medical Monitor's discretion.

3.2. Dosage And Administration

On Day 1 (baseline) of Main Study, eligible patients will be randomized 16:8:6:24:15 to Placebo QW, Placebo Q2W, pegozafermin 15 mg QW, 30 mg QW and 44 mg Q2W, respectively. IP will be administered over 24 weeks (includes 24 administrations in QW dose regimen and 12 administrations in Q2W dose regimen). Patients will undergo a second liver biopsy at the Week 24 visit (window of -7 days to +14 days). Liver biopsies will be read centrally by liver pathologists who are blinded to the treatment group assignment.

Patients completing the Main Study will continue for an additional 24 weeks in the Extension Study. The Extension Study will commence at Main Study Week 24 visit. Patients randomized to placebo QW in the Main Study will be re-randomized 1:1 to pegozafermin 30 mg QW (n=21) or placebo QW (n=21) and receive the re-randomized treatment beginning at the Week 26 visit (which may be an in-clinic or a remote home visit by healthcare professional who will administer the IP.). All other patients will continue to receive the same treatment regimen in the Extension Study that they received during the Main Study. IP will be administered over 24 additional weeks (includes 24 administrations in QW dose regimen and 12 administrations in Q2W dose

regimen), for approximately 48 weeks of treatment over the entirety of the study (Main and Extension). Specifically-identified Sponsor personnel will not be blinded; however, subjects, investigators and site staff will remain blinded to IP (placebo or pegozafermin). Dose regimen (QW or Q2W) will be known to all parties in the Extension Study.

Table 1: Number of Subjects and Intervention Groups

Treatment Group	Dose	Frequency	Route of Administration	Number of subjects
Placebo	-	QW	SC injection	42*
Placebo	-	Q2W	SC injection	22
Pegozafermin	15 mg	QW	SC injection	16
Pegozafermin	30 mg	QW	SC injection	64
Pegozafermin	44 mg	Q2W	SC injection	40

* At Week 24, subjects randomized to placebo QW in the Main Study will be re-randomized 1:1 to pegozafermin 30 mg QW (n=21) or placebo QW (n=21).
*Abbreviations: QW, weekly; Q2W, every 2 weeks; SC, subcutaneous.

3.3. Randomization and Stratification

Main Study: Patients fulfilling all of the qualifying criteria and none of the exclusionary criteria were initially centrally randomized 2:1:3:3:3 to Placebo QW, Placebo Q2W, pegozafermin 15 mg QW, 30 mg QW and 44 mg Q2W, respectively on Day 1 and enter a double-blind, 23-week treatment period. The randomization will be stratified by Type 2 diabetes mellitus (T2DM) status (yes vs no) and fibrosis stage (F2 vs F3). In April 2022, the randomization ratio was updated to 16:8:6:24:15 with number of 15 mg QW subjects capped at ~20 subjects. For the purposes of stratification, patients will be considered to be T2DM status positive if any of the following criteria is met: current medical history of T2DM, current use of anti-glycemic medication(s) for T2DM, or screening laboratory values of ≥6.5% for glycated hemoglobin (HbA1c) or ≥126 mg/dL for fasting plasma glucose. The proportion of subjects enrolled with magnetic resonance imaging-estimated proton density fat fraction (MRI-PDFF) <8% may be limited at the Sponsor's discretion.

In the Extension Study, patients randomized to placebo QW in the Main Study will be re-randomized 1:1 to pegozafermin 30 mg QW (n=21) or placebo QW (n=21) after completing the Main Study at Week 24. The Extension Study is a single-blinded, 24-week treatment period.

All patients will be centrally assigned to randomized IP using an Interactive Response Technology (IRT). IP will be dispensed at the study visits as summarized in the SoA.

3.4. Blinding

In the Main Study, patients, investigators and all site personnel, as well as Sponsor will be blinded to treatment assignment and to IP (placebo or pegozafermin) throughout the Main Study. In the Extension Study, specifically-identified Sponsor personnel will not be blinded; however, subjects, investigators and site staff will remain blinded to IP (placebo or pegozafermin). However, the dose regimen (QW or Q2W) will be known to all parties involved in both the Main and the Extension Study. Liver biopsies will be read centrally by liver pathologists who are blinded to the treatment group assignment and study visit. Results of the following assessments post Study Day 1, including but not limited to, liver biopsy, magnetic resonance imaging (MRI),

PK and anti-drug antibodies (ADA), will be considered as potential unblinding information and will remain blinded throughout the study, unless specified otherwise.

Specifically-identified Sponsor personnel will be unblinded for decision-making purposes and interactions with the United States Food and Drug Administration (FDA) after all subjects complete the Week 24 assessments in the Main Study. Sponsor personnel responsible for day-to-day study operations will remain blinded, including the Sponsor and CRO Medical Monitor. Details on unblinding the primary analysis are described in the study blinding and unblinding plan prior to unblinding the WK24 results of this study.

Other specified personnel or independent vendors may be unblinded based on their study role. These individuals include those who analyze PK or immunogenicity samples in the bioanalytical laboratories, manage the unblinded data, manage IP inventory, manage expedited reporting of suspected unexpected serious adverse events (SUSARs), and who conduct unblinded analyses for the Data Monitoring Committee (DMC). DMC members will have access to unblinded data for the purposes of reviewing unblinded overall safety results.

3.5. Sample Size Considerations

The planned sample size is chosen to sufficiently demonstrate the treatment effect in histological response at Week 24 and to enable characterization of the treatment effect size and variability around the histological response to support planning of statistical analyses and powering for the Phase 3 study and to provide adequate dose response information to support Phase 3 program dose selection.

Approximately 184 subjects will be randomized in a ratio of 16:8:6:24:15 to Placebo QW (N=42), Placebo Q2W (N=22), pegozafermin 15 mg QW (N=16), 30 mg QW (N=64), and 44 mg Q2W (N=40) in each pegozafermin group, respectively.

The sample size is selected based on a placebo response rate of 15% for NASH resolution without worsening of fibrosis and a placebo response rate of 20% for improvement of fibrosis ≥1 stage without worsening of NASH. These placebo response rates are supported by a comprehensive review on placebo histological response rates in previously reported NASH studies and recent meta-analyses (Han, 2019 Rinella, 2019; Drenth, 2020; Mesenbrink, 2020; Nikoolenejad, 2020). The anticipated treatment effect is 30% for both histological endpoints, and is supported by the observed pegozafermin effects on both histological endpoints and in reducing MRI-PDFF and alanine aminotransferase (ALT) in a Phase 1b/2a study BIO89-100-002, the relationship between MRI-PDFF and ALT reduction to histological responses (Hoofnagle, 2013; Seko, 2015; Loomba, 2019; Loomba, 2020a, Loomba, 2020b; Loomba, 2020c), and the observed histological responses in molecules with similar mechanism of action with treatment shorter than or close to 24 weeks (Akero Therapeutics Inc, 2020; Harrison, 2020a; Harrison, 2020b). Hence, the assumed pegozafermin response rates are 45% and 50% for NASH resolution and fibrosis improvement, respectively.

With these assumptions, the planned sample size of 64 patients per 30mg QW and placebo group will be able to detect the treatment differences with 94% power for NASH resolution and 92% for fibrosis improvement at a two-sided nominal 0.05 significance level and accounting for a dropout rate of up to 15%. For the comparison of the treatment difference between 44mg Q2W group (N=40) and placebo group (N=64), the power will be 87% for NASH resolution and 83% for fibrosis improvement.

3.6. Primary Analysis

The primary analyses will be conducted after all patients have completed the Week 24 assessments or prematurely discontinued from the study. All Main Study endpoints listed in Section 4.1 will be analyzed and reported.

The study blinding will not be broken for the study team (subjects, investigators/site personnel and Sponsor) until the final analysis. Specifically-identified sponsor personnel will be unblinded to summary results of the primary analyses for internal decision-making purpose. The list of Sponsor personnel unblinded to the summary results will be recorded in the study blinding and unblinding plan. Subjects and investigators/site personnel will remain blinded for the extension safety study.

3.7. Final Analysis

After all patients have completed the study, i.e., the end of study visit, and the data for all patients have been cleaned and finalized, the study will be unblinded and the final analysis will be performed.

Data Monitoring Committee (DMC)

An independent DMC will periodically review overall unblinded safety data.

Based on these reviews of emerging results, the DMC will recommend continuation, modification of the protocol, or termination of the study.

Composition of the DMC, meeting structure, schedule, and procedures, the content and format of DMC reports, and other relevant details will be determined in consultation with DMC members and detailed in a separate DMC charter.

4. STUDY ENDPOINTS AND COVARIATES

4.1. Main Study Endpoints

4.1.1. Primary Efficacy Endpoints

- [a] Proportion of patients with NASH resolution[2] without worsening of fibrosis[3] at Week 24 compared to baseline
- [b] Proportion of patients achieving improvement of fibrosis ≥1 stage without worsening of NASH[4] at Week 24 compared to baseline.

4.1.2. Key Secondary Efficacy Endpoints

- [c] Proportion of patients with at least a 2-point improvement in NAS and no worsening of fibrosis at Week 24 compared to baseline
- [d] Proportion of patients with NASH resolution AND fibrosis improvement ≥1 stage at Week 24 compared to baseline.
- [e] Proportion of subjects with ≥ 2-point improvement in NAS score AND are MRI-PDFF responders[5] AND ALT responders[6] at Week 24 compared to baseline.

Table 2 is a summary of meeting selected criteria for each primary and key secondary efficacy endpoint.

Table 2: Primary and Key Secondary Endpoints Criteria

Criteria (at Week 24)	[a]	[b]	[c]	[d]	[e]
NASH resolution	Y			Y	
No NASH worsening		Y			
No fibrosis worsening	Y		Y		
Fibrosis ≥ 1 stage improvement		Y		Y	
≥2 points NAS improvement			Y		Y
MRI-PDFF responder					Y
ALT responder					Y

[2] Resolution of NASH includes the total absence of ballooning (score=0) and absent or mild inflammation (score 0 to 1).
[3] Worsening of fibrosis is defined as progression of fibrosis ≥ 1 stage.
[4] Worsening of NASH is defined as increase in NAS for ballooning, inflammation, or steatosis.
[5] ≥30% reduction from baseline in liver fat by MRI-PDFF.
[6] ≥17 U/L or ≥30% reduction from baseline in ALT.

Efficacy Assessments: NAS Score and NASH CRN Fibrosis Staging

The NASH CRN semi-quantitative scoring of histological features (steatosis, ballooning degeneration, and lobular inflammation) which together comprise the unweighted sum to produce the NAFLD Activity Score (NAS) and the NASH CRN Fibrosis staging system are as follows:

	Steatosis	Ballooning	Lobular Inflammation
Grade 0	≤ 5%	None	No foci
Grade 1	>5% and ≤ 33%	Few Balloon cells	<2 foci/200x
Grade 2	>33% and ≤ 66%	Many cells/prominent ballooning	2-4 foci/200x
Grade 3	>66%		>4 foci/200x

NAS total score ranges from 0 – 8, which is the sum of Steatosis, Ballooning, and Lobular Inflammation.

Stage (Score)	Fibrosis
Stage 0 (0)	None
Stage 1 (1)	Perisinusoidal or periportal
Stage 1A (1)	Mild, zone 3, perisinusoidal
Stage 1B (1)	Moderate, zone 3, perisinusoidal
Stage 1C (1)	Portal/periportal
Stage 2 (2)	Perisinusoidal and portal/periportal
Stage 3 (3)	Bridging fibrosis
Stage 4 (4)	Cirrhosis

NASH CRN fibrosis stage ranges from F0 (without fibrosis) to F4 (cirrhosis) per NASH CRN system.

4.1.3. Other Secondary Efficacy Endpoints

- Absolute change and percentage change from baseline to Week 12 and Week 24 in:
  - Hepatic fat fraction by MRI-PDFF
  - ALT
  - N-terminal type III collagen propeptide (Pro-C3)
  - Adiponectin
  - Serum triglycerides
  - High density lipoprotein cholesterol (HDL-c)
  - Non-HDL cholesterol (Non-HDL-c)
  - Low density lipoprotein-cholesterol (LDL-c)
  - Glycated hemoglobin (HbA1c)

4.1.4. Pharmacokinetics PK Endpoints

Trough concentration of pegozafermin.

4.1.5. Safety Endpoints

- Frequency and severity of TEAEs and SAEs
- Number of subjects who discontinued due to TEAEs and due to related TEAEs
- Incidence and shifts of clinically significant physical examination findings, ECG data and laboratory abnormalities; safety laboratory evaluations include hematology, blood biochemistry and urinalysis, serum, salivary, and urinary cortisol as appropriate
- Change from baseline in
  - Insulin-like growth factor 1 (IGF-1)
  - Bone biomarkers: Carboxy-terminal collagen crosslinks (CTX), N-terminal propeptide of type 1 collagen (P1NP) and osteocalcin
  - Thyroid stimulating hormone (TSH)
- Absolute and % change from baseline in lumbar spine, total hip, and femoral neck bone mineral density (BMD) as assessed by dual X-ray absorptiometry (DXA)
- Incidence and characteristics of anti-drug antibodies (ADA) and neutralizing antibody (NAb) after dosing (e.g., titer and binding specificity, to the FGF21 and polyethylene glycol [PEG] part of pegozafermin)
- Impact of the presence of ADAs on serum pegozafermin concentrations and clinical safety

4.1.6. Exploratory Endpoints

4.1.6.1. Change from baseline to Week 24 in the following clinical parameters

4.1.6.1.1. Histological assessments

Proportion of subjects at Week 24 with

- Fibrosis improvement ≥2 stage without worsening of NASH
- NASH resolution without worsening of fibrosis OR improvement of fibrosis ≥1 stage without worsening of NASH
- NASH resolution without worsening of fibrosis and ≥ 2-point improvement in NAS score
- Improvement of fibrosis ≥1 stage without worsening of NASH and ≥ 2-point improvement in NAS score
- At least a 2-point improvement in NAS with at least a 1-point improvement in ballooning or lobular inflammation, and no worsening of fibrosis
- Decrease >1 stage in fibrosis score

  - At least 2-point improvement in NAS score AND are MRI-PDFF responders
  - Fibrosis improvement ≥1 stage without worsening of NASH AND are MRI-PDFF responders AND ALT responders
  - Fibrosis improvement ≥1 stage without worsening of NASH AND are MRI-PDFF responders
  - Fibrosis improvement ≥1 stage without worsening of NAS score
  - Fibrosis improvement ≥1 stage and cT1 reduction by ≥80 msec
  - NASH resolution without worsening of fibrosis AND are MRI-PDFF responders AND ALT responders
  - NASH resolution without worsening of fibrosis AND are MRI-PDFF responders
  - NASH resolution and cT1 reduction by ≥80 msec
- Change from baseline in:
  - NAS total score
  - Fibrosis score

In MRI-PDFF responders

Proportion of patients with

  - Fibrosis improvement ≥2 stage without worsening of NASH
  - NASH resolution without worsening of fibrosis, OR improvement of fibrosis ≥1 stage without worsening of NASH
  - NASH resolution AND fibrosis improvement ≥1 stage
  - At least a 2-point improvement in NAS with at least a 1-point improvement in ballooning or lobular inflammation AND no worsening of fibrosis
  - At least a 2-point improvement in NAS with at least a 1-point improvement in ballooning or lobular inflammation AND fibrosis improvement ≥1 stage

Change from baseline in NAS score.

- Artificial intelligence (AI)-based histological analysis (PathAI).

4.1.6.1.2. Imaging assessments

- Hepatic fat content with
  - Percentage of subjects with ≥30% relative reduction in hepatic fat fraction as assessed by MRI-PDFF at Weeks 12 and 24
  - Percentage of subject with ≥50% relative reduction in hepatic fat fraction as assessed by MRI-PDFF at Weeks 12 and 24
  - MRI-PDFF ≤5% at Weeks 12 and 24

- Absolute and percent change from baseline in hepatic fibro-inflammation as measured by Iron-corrected T1 (cT1) imaging
- Percentage of subjects with ≥80 msec relative reduction in hepatic fibro-inflammation as measured by Iron-corrected T1 (cT1) imaging at Weeks 12 and 24
- Absolute and percent change from baseline in Fibroscan Vibration-controlled transient elastography (VCTE) score (liver stiffness measurement) and CAP (steatosis)
- Absolute and percent change from baseline in liver and spleen volume
- Absolute and percent change from baseline in pancreatic fat percentage

4.1.6.1.3. Anthropometric assessments

- Body weight
- Waist circumference
- Waist/hip ratio

4.1.6.1.4. Laboratory parameters

- Percentage of subjects with ≥17 U/L decrease in ALT
- Percentage of subjects with ≥30% decrease in ALT
- AST
- Gamma-glutamyl transpeptidase (GGT)
- Alkaline phosphatase (ALP)
- Fasting glucose
- C-peptide
- Fasting insulin
- Adipo-IR index (fasting free fatty acids x fasting insulin)
- Homeostatic model assessment for insulin resistance (HOMA-IR)
- Enhanced liver fibrosis (ELF) score
- Fibrosis-4 (FIB-4) index
- Absolute and percent change from baseline in HbA1c in subjects with baseline HbA1c ≥ 6.5%

4.1.6.2. Change from baseline PRO scores at Weeks 12 and 24 in

- Chronic Liver Disease Questionnaire - NAFLD-NASH (CLDQ NAFLD-NASH)
- Questionnaire for NASH (WPAI-NASH)
- Europe Quality of Life Group 5 dimension 5 level questionnaire (EQ-5D-5L)

- Liver-related pain and discomfort questionnaire
- The appetite sensations visual analog scale (VAS)

4.2. Extension Study Endpoints

4.2.1. Primary Endpoints

- Frequency and severity of TEAEs and SAEs at Week 48

4.2.2. Secondary Endpoints

- Absolute change and percent change from baseline at Week 48 in liver parameters:
  - Hepatic fat fraction by MRI-PDFF
  - ALT
  - Pro-C3
- Trough concentration of pegozafermin

4.2.3. Safety Endpoints

- Number of subjects who discontinued due to TEAEs and due to related TEAEs
- Incidence and shifts of clinically significant physical examination findings, ECG data and laboratory abnormalities; safety laboratory evaluations include hematology, blood biochemistry and urinalysis, serum, salivary, and urinary cortisol as appropriate
- Insulin-like growth factor 1 (IGF-1)
- Bone biomarkers: Carboxy-terminal collagen crosslinks (CTX), N-terminal propeptide of type 1 collagen (P1NP) and osteocalcin
- Thyroid stimulating hormone (TSH)
- Absolute and % change from baseline in lumbar spine, total hip, and femoral neck bone mineral density (BMD) as assessed by dual X-ray absorptiometry (DXA)
- Incidence and characteristics of anti-drug antibodies (ADA) and neutralizing antibody (NAb) after dosing (e.g., titer and binding specificity, to the FGF21 and polyethylene glycol [PEG] part of pegozafermin)
- Impact of the presence of ADAs on serum pegozafermin concentrations and clinical safety
- Levels of endogenous FGF21 at the immunogenicity follow-up visit(s) compared to baseline in NAb positive subjects

4.2.4. Exploratory Endpoints

4.2.4.1. Mean and percent change from baseline at Weeks 38 and 48 in the following metabolic parameters:

- Adiponectin
- Serum triglycerides
- HDL-c, Non-HDL-c, LDL-c, HbA1c.

4.2.4.2. Change from baseline in the following clinical parameters:

4.2.4.2.1. Imaging assessment

- Change in hepatic fibro-inflammation as measured by cT1 imaging
- Change in FibroScan VCTE score (liver stiffness measurement) and CAP (steatosis)

4.2.4.2.2. Anthropometric assessments for

- Body weight
- Waist circumference
- Waist/hip ratio.

4.2.4.2.3. Laboratory parameters

- Percentage of subjects with ≥17 U/L decrease in ALT
- Percentage of subjects with ≥30% decrease in ALT
- AST, GGT, ALP
- Fasting glucose
- C-peptide
- Fasting insulin
- Adipo-IR index (fasting free fatty acids x fasting insulin)
- HOMA-IR
- ELF score
- FIB-4 index

4.2.4.3. Change from baseline PRO scores at Weeks 48 in

- CLDQ NAFLD-NASH
- WPAI-NASH
- EQ-5D-5L
- Liver-related pain and discomfort questionnaire

- The appetite sensations VAS

A cross table of specified criteria and each exploratory endpoint is provided in (Main Study exploratory proportion endpoints).

4.3. Subgroups

Subgroup analysis will be performed in the following baseline subgroups for primary efficacy endpoints. If the percentage of patients within a subgroup is less than 33% of overall subjects and the number of patients in the subgroup is lower than 15, only descriptive analysis will be performed.

- Age group (21 - <65, ≥65)
- Race (White, Non-white)
- Sex (Male, Female)
- Baseline BMI group (<30, 30 - <35, ≥35 kg/m2)
- Baseline T2DM (Yes, No)
- Baseline Fibrosis stage (F2, F3)
- Baseline MRI-PDFF (<median, ≥median)
- Baseline ALT (normal, >ULN)
- Baseline NAS score (<median, ≥median)

5. DEFINITIONS

5.1. Baseline Definition

For efficacy, safety (except ALT, AST, lipids, and vital signs), and biomarkers each subject's baseline value for that measurement will be defined as the last non-missing value collected on or before the day of first study drug dose, or randomization date + 5 days if subjects have never been dosed. Other baseline values, such as demographics and pertinent medical history etc., is defined as last available value before randomization date.

The definition of baseline of lipid panel labs is the average of last non-missing screening fasting values and Study Day 1 value (Pre-dose, fasting).

The definition of baseline vital signs is the average of the last non-missing screening values and Study Day 1 value (Pre-dose). If there is Case Report Form (CRF) average value on duplicate measurements of BP and/or Pulse on Study Day 1, only the individual values will be included in the baseline derivation.

Baseline ALT and AST were defined as below:

Table 3: Baseline Definition for ALT and AST Values

ALT/AST Screening Assessments			Day 1 ALT/AST Assessment	Baseline Value
Assessment 1	**Assessment 2**	**Assessment 3 (if applicable)**		
Normal	Normal	Not applicable	Any	Average of Assessment 1, Assessment 2 and Day 1 (3 tests)
Normal	Abnormal and ≤40% increase from Assessment 1	Not applicable	Any	Average of Assessment 1, Assessment 2 and Day 1 (3 tests)
Normal	Abnormal and >40% increase from Assessment 1	Normal or ≤40% increase from Assessment 1	Any	Average of Assessment 1, Assessment 2, Assessment 3 and Day 1 (4 tests)
		Abnormal and >40% increase from Assessment 1	Not applicable, subject excluded	Not applicable, subject excluded
Abnormal	≤40% increase from Assessment 1	Not applicable	Any	Average of Assessment 1, Assessment 2 and Day 1 (3 tests)
Abnormal	>40% increase from Assessment 1	≤40% increase from Assessment 1	Any	Average of Assessment 1, Assessment 2, Assessment 3 and Day 1 (4 tests)
		>40% increase from Assessment 1	Not applicable, subject excluded	Not applicable, subject excluded

5.2. Actual Treatment Group

Primary Analysis for Main Study

If a subject receives only placebo doses, then their actual treatment group will be placebo group. If a placebo subject incorrectly received at least one pegozafermin active dose, then they will be counted as the pegozafermin active treatment group in the highest dose group received for safety analysis. If a pegozafermin subject receives at least one dose of pegozafermin active treatment, then the most frequent dose received will be considered as the actual treatment group.

Final Analysis

In the final analysis, the actual treatment arm will be assigned following the same rule as in the primary analysis for Main Study for subjects who were not re-randomized in the Extension study.

The actual treatment group will be assigned as pegozafermin in the highest dose level if subject switched from Placebo to pegozafermin in the Extension Study. However, subjects who switched the treatment in the Extension Study will be summarized under "Placebo (main) and

Pegozafermin 30 mg QW (Extension)" group as a separate column in the final safety summaries and efficacy analysis.

5.3. Study Day

The day a subject receives their first dose of IP will be considered as Study Day 1.

If a subject is randomized but never dosed, study day will be calculated using the randomization date and randomization date will be set to Study Day 1.

If date of interest occurring on or after the relative date, relative day is calculated as: date of interest – relative date + 1, else if date of interest occurring prior the relative date, relative day is calculated as: date of interest – relative date. Data will be presented on listings in order of site number/subject number, assessment date and assessment (in order collected on CRF, unless specified otherwise). Dates will be presented in the format of YYYY-MM-DD.

5.4. Prior Medication, Concomitant Medication / Procedures

Any medication that was stopped prior to the Study Day 1 is considered as prior medication.

Any medication/procedure that is taken any day on or after Study Day 1 is considered as concomitant medication/procedure. This includes medications/procedures that were started prior to administration of first dose and that were continued to be taken after Study Day 1.

5.5. Treatment Emergent Adverse Event (TEAE)

TEAE during the Main Study

All AEs started or worsened within the first dose of IP up to the min of (the last dose date before the completion of all Week 24 assessments in the Main Study IP+28 days, the date all Week 24 assessments completed, Main Study cut-off date) will be considered as TEAEs during the Main Study. For subjects who withdraw consent from the Main Study, the end date of the TEAE reporting period should be the earlier of the withdrawal consent date and the date of the last dose of the IP + 28 days.

TEAE during the Study

All AEs that started or worsened on or after the first dose of study IP up to the last dose of the study IP+28 days will be considered as TEAEs.

For subjects who withdraw consent from the study, the end date of the TEAE reporting period should be the earlier of the withdrawal consent date and the date of the last dose of the IP + 28 days.

6. ANALYSIS POPULATIONS

For purposes of analysis, the following populations are defined:

Population	Description
Randomized Analysis Set	All enrolled subjects who are assigned a randomization number in the study.
Full Analysis Set (FAS)	All enrolled subjects with confirmed fibrosis stage F2 or F3 and NAS ≥4 at baseline per independent review by a 3-pathologist panel who are eligible and assigned a randomization number in the study and received at least 1 dose of IP. For analysis purposes, subjects will be analyzed according to the treatment they were randomized to regardless of actual treatment received.
Safety Analysis Set	All randomized subjects who receive at least 1 dose of IP. In this population, subjects will be summarized based upon the IP actually received, regardless of the IP to which they were randomized.
PK Analysis Set	All subjects in the FAS who have sufficient data to adequately characterize the trough serum pegozafermin concentrations and have no other events or protocol violations that would adversely affect results, such as not completing the full dose. The analysis population for any population PK modeling may be defined separately in a population PK data analysis plan.
MRI-PDFF Analysis Set	All subjects in the Full Analysis Set who have a baseline and at least one follow up MRI-PDFF assessment.

Primary Analysis

- Patient dispositions and screen failure summary will be presented for all subjects screened.
- Baseline characteristics, protocol deviations, medical histories and concomitant medications will be assessed for Randomized Analysis Set.
- All primary, secondary, and exploratory efficacy endpoints of Main Study will be assessed for Full Analysis Set.
- Exposure and safety assessments will be analyzed based on Safety Analysis Set.
- PK endpoints will be displayed and analyzed for all subjects with applicable data in the PK Analysis Set.

Final Analysis

- Protocol deviations and concomitant medications will be summarized based on Randomized Analysis Set including data in Extension Study.
- Exposure and safety assessments will be summarized based on Safety Analysis Set including data in Extension Study.
- PK endpoints will be displayed and analyzed for all subjects with applicable data in the PK Analysis Set.

6.1. Handling of Missing and Incomplete Data

6.1.1. Missing Dates

For the missing or partially missing dates for the adverse event, medical history, prior/concomitant medication and procedures, the missing date will be imputed following the rules below.

6.1.1.1. Adverse Events

- The missing day of onset of an AE will be set to:
  - First day of the month that the event occurred, if the onset YYYY-MM is after the YYYY-MM of first study treatment
  - The day of the first study treatment, if the onset YYYY-MM is the same as YYYY-MM of the first study treatment
  - The minimum date of the informed consent date and the first day of the month that the event occurred, if the onset YYYY-MM is before the YYYY-MM of the first treatment.
- The missing day of resolution of an AE will be set to:
  - The last day of the month of the occurrence. If the subject died in the same month, then set the imputed date as the death date.
- If the onset date of an AE is missing both the day and month, the onset date will be set to:
  - January 1 of the year of onset, if the onset year is after the year of the first study treatment
  - The date of the first treatment, if the onset year is the same as the year of the first study treatment
  - The date of informed consent, if the onset year is before the year of the first treatment
- If the resolution date of an AE or end date of an IP is missing both the day and month, the date will be set to:
  - December 31 of the year of occurrence. If the subject died in the same year, then set the imputed date as the death date.

6.1.1.2. Prior/concomitant medication/procedure

- If data indicated the stop date of a therapy is before the date of first dose, then no need to impute the start/stop date of this therapy
- The missing day for the start date of a therapy (for which a start month and year are entered) will be set to the first day of the month that the event occurred.
- The missing day for the end date of a therapy (for which an end month and year are entered) will be set to the last day of the month of the occurrence.
- If the start date of a therapy is missing both the day and month (but includes a year in which the therapy was started), the onset date will be set to January 1 of the year of onset.
- If the end date of a therapy is missing both the day and month (but includes a year in which the therapy ended), the date will be set to December 31 of the year in which the therapy ended.
- If the start date of a therapy is unknown (missing the day, month, and year) and the end date is not a complete date then the start date will be set to either the imputed partial end date or the date of the first study visit, whichever is earlier.
- If the start date of a therapy is unknown (missing the day, month, and year), the end date is a complete date, and the end date is after the date of the first study visit, then the start date will be set to the date of the first study visit. Otherwise, the start date will be set to the end date of the therapy.
- If the end date of a therapy is null and the start date is not a complete date then the end date will be set to the study end date.
- If the end date of a therapy is null and the start date is a complete date
  - and the start date is prior to the study end date then the end date will be set to the study end date.
  - otherwise, the end date will be set to the start date of the therapy.

6.1.2. Missing for Fasting Status

If fasting status is missing or incomplete, then fasting status will be considered as “not fasting.”

6.1.3. Missing for AE Relationship Status

If AE relationship status is missing or incomplete, then AE relationship status will be considered as “related.”

6.2. Analysis Visit Windows

In general, scheduled visits from randomization (except for early terminated subjects) will be used as analysis visit for descriptive analysis tables and listings. Unscheduled visits, ET visit, and EOS visit will be mapped according to the actual study day and analysis visit window defined in Table 4.

Scheduled visit will be selected for analysis/summaries if has valid assessment result. If a scheduled visit is missing, the unscheduled visit that is closest to the target study day will be used for analysis purpose.

Table 4: General Analysis Visit Window

Study Period	Target Study Weeks	Target Study Day	Analysis Visit Window (Days)
Screening Period	-12 to 0	--	--
Main Study	--	1	--
	4	29	2 to 43
	8	57	44 to 71
	12	85	72 to 99
	16	113	100 to 141
	24	169	142 to min (the date all Week 24 assessments completed, Main Study cut-off date)
Extension Study	26	183	•
	30	211	(W24 End date+1) to 239
	38	267	240 to 302
	48	337	303 to 351
Follow-up Period	ET	--	--
	51/EOS	358	>= 352

Week 26 is the start visit for the extension study dosing, no need to be included in the visit-based summary tables if no data is collected.

For parameters with scheduled visit(s) at Week 12 and Week 24, or Week 24 only, the analysis window will be adjusted such that:

- The first post-baseline scheduled visit will start at day 2. Other visits start from the end of previous visit + 1 day.
- The end of each analysis visit window will be middle to the next scheduled visit (If an even number of days exists between two consecutive visits then the end date will be taken as the midpoint value minus 1 day).

6.3. Software

All data processing, summarization and analyses will be performed using SAS Version 9.4 or later of the SAS ® statistical software package.

7. STATISTICAL METHODS OF ANALYSES

7.1. General Considerations

The primary analysis will include data up to the min of (28 days after the last dose date before the completion of all Week 24 assessments, the date all Week 24 assessments completed, Main Study cut-off date).

At final analysis, safety summaries including extent of exposure will report data from both Main Study and Extension Study, based on safety analysis set.

Continuous variables will be summarized by number of patients, mean, standard deviation (SD)/ standard error (SE), median, minimum, and maximum values. Minimum and maximum will be presented to the same number of decimal places as reported/collected. One additional decimal place will be presented for mean and median. Two additional decimal places will be presented for SD/ SE up to a max of three decimal places.

Categorical variables will be summarized by number and percentage of patients. Percentage will be presented to one decimal place. The number of missing values will be presented as a separate category with no percentage, but only if 1 or more patients are missing data for the summary. Otherwise, all categories will be presented (even if no patients are counted in the category). Counts of zero in any category will be presented without percentage.

For analysis purpose, placebo QW and placebo Q2W will be pooled except for exposure summaries and pre-specified supportive analyses.

All statistical tests will be 2-sided and tested at a statistically significant level of 0.05 without adjustment for multiplicity. Confidence intervals will be 2-sided 95%, unless stated otherwise. Two-sided p-values will be presented with four decimal places and values less than 0.0001 will be presented as <.0001.

Both reported units (CN) and standard units (SI) will be presented in listings. Only CN units will be used for tables and figures, unless otherwise specified. Insulin will be presented with conventional unit of µIU/mL.

All applicable analyses for the primary analysis of the Main Study will be presented as:

<Description>	Placebo Pooled	Pegozafermin 15 mg QW	Pegozafermin 30 mg QW	Pegozafermin 44 mg Q2W

A total column will be presented for the disposition, demography, protocol deviation, medical history, prior/concomitant medication/procedure, and other subject information summaries.

The listings will only present non-imputed data unless otherwise specified.

7.2. Patient Disposition

Primary Analysis for Main Study

The number of patients screened and the number (percentage) of patients who failed screening (i.e., patients who consented to participate in the clinical study but were not subsequently randomized) and the reasons for screen failure will be summarized. The number of patients in each analysis set will be summarized by treatment group and overall.

In addition, the number of patients who discontinued the study IP and withdrew from the study will be summarized with treatment/study discontinuation reasons (including discontinuation due to COVID-19 pandemic).

The information including demography, screen failure reasons, eligibility criteria, end of treatment along with reasons, end of study along with reasons will be listed.

Final Analysis

In addition to repeat the disposition table of the Main Study following the final analysis layout, a separate summary table will be provided for subjects who switched the treatment in the Extension Study. The number (percentage) of patients who received at least one dose of pegozafermin in Extension Study, withdrew from the study, and discontinued the pegozafermin treatment along with the discontinuation reasons will be summarized.

The information including informed consent to Extension Study date, re-randomization, end of pegozafermin treatment dates, end of study dates, and discontinuation reasons will be listed.

7.3. Protocol Deviations

A full list of protocol deviations will be compiled and reviewed by the clinical team to identify Important versus non-important protocol deviations before the primary analysis and before the final database lock. For deviations at study entry, patients will be assessed against the inclusion and exclusion criteria of the protocol. For on-study deviations, compliance with the protocol will be examined using blinded review of the database regarding prohibited therapies, and timing and availability of planned assessments.

Patients having important protocol deviations will be summarized using counts and percentages by deviation category and coded deviation term based on the Full Analysis Set for Main Study and will be re-generated at final analysis including protocol deviations occurring during the Extension Study.

A listing of all protocol deviations will be provided with treatment period flag (Main Study or Extension Study). Important protocol deviations will be flagged in the listing.

7.4. Demographic and Baseline Characteristics in Main Study

The following demographic and baseline characteristics will be summarized by treatment group, as well as overall, for Randomized Analysis Set:

- Age in years and by age group (21-<65, >=65)
- Gender
- Race
- Ethnicity
- Height (cm), weight (kg), weight group, body mass index (kg/m^2), body mass index (BMI) group (< 25, 25 -< 30, ≥ 30 kg/m^2), waist and hip circumferences (cm) and waist to hip ratio
- Systolic and diastolic blood pressure (mmHg)
- Randomization factors (T2DM status and fibrosis stage)
- Screening HbA1C >= 6.5% (Yes/ No)
- Baseline HbA1C >= 6.5% (Yes/ No)
- Fasting plasma glucose >= 126 mg/dL (Yes/ No)

- Liver biopsy results (fibrosis stage, steatosis score, ballooning degeneration score, lobular inflammation score, NAS)
- MRI-PDFF (>=8%), cT1, ELF > 9.8 (Yes/No), Fibroscan VCTE kPa and CAP score
- ALT, Pro-C3, Adiponectin, serum triglycerides, HDL-c, Non-HDL-c, LDL-c, HBA1c.
- Estimated Glomerular Filtration Rate (eGFR) category (normal: ≥90 mL/min/1.73m2; mild Renal Impairment: 60-89 mL/min/1.73m2; moderate Renal Impairment: 30-59 mL/min/1.73m2, and severe Renal Impairment: 15-29 mL/min/1.73m2)
- History of hypertension
- History of lipid disorder
- History of obesity
- History of menstrual disorder (for females)
- History of COVID-19 and complications.

7.5. Medical History

General medical history and background therapy will be summarized at primary analysis by treatment group, as well as overall, for FAS population and presented in a by-subject listing.

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 24.0 or higher.

7.6. Prior and Concomitant Medication/Procedure

Medications will be coded using World Health Organization-Drug dictionary (WHO-DD), March 2021 or later.

The frequencies of use of prior/ concomitant medications will be summarized by treatment group for FAS according to Anatomical Therapeutic Chemical (ATC) class and preferred term.

Prior and concomitant medications/ procedure will be listed for each subject.

7.7. Extent of Exposure and treatment compliance

The length of exposure, number of actual drug doses and number of missed doses, total dose administered (mg), and study treatment compliance will be summarized by actual treatment group for the Main Study and for the Entire Study.

The IP administration and compliance data, including reasons for poor compliance, will be listed.

Main Study

Length of Main Study exposure in days will be derived as:

- Length of Main Study exposure (days) for QW dosing = (date of last dose in Main Study) – (date of first dose in Main Study) + 7;
- Length of Main Study exposure (days) for Q2W dosing = (date of last dose in Main Study) – (date of first dose in Main Study) + 14.

Total dose administered (mg) in Main Study will be calculated as number of doses received x dosage (mg) of the investigational product contained in each dose.

The Main Study compliance = (total doses administered in Main Study /total doses expected in Main Study) x 100%. Total doses expected is defined as the total number of doses protocol defined until the end of Main Study treatment. If the dose of a kit is not fully administrated, the dosage will be imputed as 0 mg for the kit.

Entire Study

The overall length of exposure (days), total dose administrated (mg), and the overall study compliance will be derived following the same logic as Main Study by the actual treatment subject received. For subjects who switched the treatment at the Extension Study, the length of exposure (days), the total dose administrated (mg), and the study compliance will be derived for placebo and pegozafermin 30mg respectively. Column "Placebo (main) and pegozafermin 30 mg QW (Extension)" will report both study drugs.

7.8. Efficacy Analysis for Main Study

The primary efficacy endpoints are (1) proportion of subjects with NASH resolution without worsening of fibrosis at Week 24 compared to baseline (2) proportion of subjects achieving improvement of fibrosis ≥1 stage without worsening of NASH at Week 24 compared to baseline. The following hypothesis will be tested for each primary endpoint:

- The null hypothesis: there is no difference in the proportion of subjects meeting the primary endpoint between the placebo and each pegozafermin dose group.
- The alternative hypothesis: the proportion of subjects meeting the primary endpoint between the placebo and each pegozafermin dose group is different.

The study will be considered successful if a given pegozafermin dose group is demonstrated to be superior to the placebo group in either of the two primary endpoints at 0.05 significance level.

Estimand Framework

As detailed in the ICH E9 (R1) addendum, the following five attributes will define the estimand framework in this trial. The primary estimand will be defined as a treatment policy (TP) estimand, which compares treatment outcomes in two randomized arms at 24 weeks post-randomization irrespective of what changes in treatment could have occurred post-randomization as a result of various intercurrent events (ICE). To implement TP strategy, it will be important that outcomes are collected and recorded after occurrence of ICE's as close to the 24 week time point, regardless of what actual treatment the patient may be receiving. Below are the 5 attributes of the primary estimand:

- Treatment conditions: Randomization to Pegozafermin (15 mg QW or 30 mg QW or 44 mg Q2W) or Placebo, regardless of ICE's and deviations from the randomized treatment and actual treatment received during or at the end of the treatment period.
- Population: Randomized subjects with fibrosis stage 2 or 3 and NAS ≥ 4 at baseline per 3-panel consensus read who received at least 1 dose of IP.
- Endpoints: NASH resolution without worsening of fibrosis at Week 24 compared to baseline; improvement of fibrosis ≥1 stage without worsening of NASH at Week 24 compared to baseline
- Intercurrent events (ICEs): Three types of ICEs are defined below.
- Population-level summary: Between treatment group difference expressed in terms of the percentage differences in achieving the endpoints.

The following types of ICEs will be documented in course of the study.

- ICE-1: Discontinuation of treatment due to lack of efficacy.
- ICE-2: Discontinuation of treatment due to adverse events (AE).
- ICE-3: All other events, i.e., protocol deviation, etc.

The primary analysis will use all observed outcomes at Week 24, regardless of actual treatment. All outcomes collected from patients experiencing this ICE after the actual ICE will be used in the analysis and missing values resulting from any ICE will be imputed assuming an imputation model informed by observed outcomes from similar patients with this type of ICE. The imputation model will include terms for the randomized treatment arm and the type of ICE(s) experienced by patients with missing outcomes, i.e., ICE-1,2,3 (as defined above) or None. The final inference following multiple imputation (MI) will be conducted using Rubin's combination rules.

Although the ICEs' defined above will be all handled by the same treatment policy strategy for the primary estimand, various sensitivity analyses (SA) will be conducted that will incorporate different strategies, depending on the type of ICE. For the purposes of sensitivity analysis, patients who experienced any of the listed ICEs (ICE-1,2,3), the outcomes for the primary efficacy endpoint will be considered missing starting from the occurrence of each ICE (even if outcomes are collected following an ICE) and then the missing outcomes will be imputed using appropriate imputation strategies.

For ICE-1, the SA will use the "return to baseline" hypothetical strategy, implying no benefits have been received for such patients from randomization until the end of the treatment period. The missing outcomes will be imputed using a relevant regression model fitted to the baseline data within each treatment arm with patient age, gender, baseline NAS score and stratification factors included as covariates.

For ICE-2, SA will be handled with the "control-based" hypothetical strategy implying that patients would have switched to the control treatment (i.e., placebo) starting from the occurrence of ICE-2 to the end of the treatment period. The missing outcomes will be imputed from a similar regression model fitted to the placebo arm alone with patient age, gender, baseline NAS score and stratification factors as covariates.

For ICE-3, SA will be handled using a hypothetical strategy that assumes that patients would have continued their assigned treatment starting from ICE-3. The missing outcomes will be imputed using the same model as for ICE-2 but fitted to each respective treatment arm.

Missing data due to reasons unrelated to the intercurrent events (ICE-1, 2, 3), e.g., missingness caused by study termination by sponsor, will be imputed using the same model as for ICE-3.

7.8.1. Primary and sensitivity analyses

The primary efficacy endpoints will be analyzed using the Full Analysis Set with confirmed fibrosis stage F2 or F3 and NAS ≥4 at baseline per independent review by a 3-pathologist panel. To evaluate the effect of BIO89-100 on liver histology, the proportions of subjects who met different histological responder criteria (defined in Section 4.1.1 and 4.1.2) at Week 24 will be summarized by treatment group based on central manual reads per central lab biopsy manual.

The Cochran-Mantel-Haenszel (CMH) test stratified by T2DM status (yes vs. no) and fibrosis stage (F2 vs. F3) will be used to compare the proportions of responders at Week 24 in each pegozafermin group versus placebo. The point estimates and 95% CI for the differences in proportions will be calculated. If two or more strata contain small cell counts (<5) that renders the stratified CMH test inappropriate, the primary analysis will use a logistic regression with treatment group indicator, stratification factors and baseline NAS score in the model.

The null hypotheses that there is no significant difference in proportion of subjects who met histological responder definition will be tested at significance level of 0.05 (two-sided) for each pegozafermin treatment group versus placebo. Type-1 error will not be adjusted for the multiplicity. Supportive analyses of each treatment QW arm vs. placebo QW arm and 44 mg Q2W arm vs. placebo Q2W arm will also be performed. The primary analysis will be repeated in all subjects with baseline and follow-up biopsies as a supportive analysis.

NAS grade of each component and NASH CRN fibrosis stage, along the shifts from baseline, will be summarized at Week 24 and different read source (central manual reads or PathAI machine reads). NAS total score and fibrosis score will be summarized descriptively at each protocol scheduled visit and different read source.

In addition to the primary analysis, standard sensitivity analyses, namely, sensitivity analyses based on the hypothetical (missingness at random) strategy, return-to-baseline imputation and control-based imputation, will be conducted. The details of these analyses are presented in Table 5. In all cases, a multiple imputation approach will be applied and imputation models will be based on a Bayesian logistic regression model with subject baseline fibrosis stage, diabetes status, age, gender and baseline NAS score included in the model. The estimated effects and associated standard errors from the individual completed data sets will be combined using Rubin's rules to produce a single inferential statement (treatment effect, standard error and p-value).

Table 5: Primary and sensitivity analyses for three types of ICEs

Analysis	ICE-1	ICE-2	ICE-3
Primary analysis	Use all collected outcomes regardless of ICEs. Impute missing outcomes based on an imputation model informed by the relevant baseline data and type of ICE ("none",1,2,3). If most subjects with ICE have missing outcomes, type of ICE covariate will be removed from the imputation model.		
Sensitivity analysis by ICE type	Set all post-baseline outcomes as missing. Impute missing outcomes based on an imputation model informed by the baseline data alone (return-to-baseline).	Set all outcomes following ICE as missing. Impute missing outcomes based on an imputation model informed by patients who did not have any of the three ICEs using only the data from the placebo arm.	Set all outcomes following ICE as missing. Impute missing outcomes based on an imputation model informed by patients who did not have any of the three ICEs using only the data from the treatment arms.

7.8.1.1. Primary analysis

The primary analysis will be carried out using an MI-based strategy that includes the following three steps:

- Step 1. Missing outcomes will be imputed a large number of times (M=100) using PROC MI based on the model outlined in Table 5 based on a logistic regression model.
- Step 2. The Cochran-Mantel-Haenszel (CMH) test stratified by T2DM status (yes vs. no) and fibrosis stage (F2 vs. F3) will be applied to each completed data set to compare the proportions of responders at Week 24 between each pegozafermin group and placebo, adjusting for the stratification factors. The point estimates and associated standard errors for the differences in proportions as well as CMH test statistics will be calculated.
- Step 3. The CMH summaries from individual completed sets will be combined using Rubin's rules to produce a single inferential statement (treatment effect, standard error, 95% confidence interval and p-value) via PROC MIANALYZE.

Note that a patient can have only one ICE (classified as ICE-1, -2, -3) or none (0). Once an ICE of specific type has occurred, the status of ICE variables remains fixed at that value until the end of the trial. All collected data are used in imputation. Imputation is done by the trial arm (TRT) but, if the data are sparce, instead of imputing by TRT, include TRT as covariate.

7.8.1.2. Sensitivity analysis

7.8.1.2.1. Per ICE type

Return-to-baseline imputation will be applied as described in Table 5 for patients who experienced ICE-1. For patients with ICE-1, set both week 24 outcomes to missing and impute the data using the baseline data alone.

Control-based imputation will be applied as described in Table 5 for patients who experienced ICE-2. Set all outcomes following ICE to missing in the treatment arm. For the placebo arm, keep only patients with no ICEs.

Treatment-continuation imputation will be applied as described in Table 5 for patients who experienced ICE-3. Set both outcomes following ICE to missing and impute the data by patients who did not have any of the three ICEs in the respective randomized arm.

7.8.1.2.2. Randomization period as an additional stratification factor

Sensitivity analysis using randomization time period (randomized before/after protocol amendment 2) as an additional stratification factor will be performed.

7.8.1.2.3. Inclusion of subjects randomized but deemed ineligible per the 3-panel

The primary analysis will be repeated by including subjects randomized and received at least 1 dose of IP but deemed ineligible per the 3-panel consensus read i.e., subjects with fibrosis stage F1 or F4 or NAS < 4 at baseline per the 3-panel consensus read.

7.8.1.2.4. Treating subjects with missing histology endpoint as non-responders

The primary analysis will be carried out by imputing a subject with missing histology endpoint as a non-responder for the endpoint.

7.8.1.3. Subgroup Analysis

The primary analyses and the completer analysis will be repeated in the subgroups, as noted in Section 4.3.

The point estimates and 95% confidence interval (CI) for the differences in proportions will be calculated for each subgroup and visualized via forest plots for primary endpoints.

7.8.2. Secondary Efficacy Analysis

The planned primary and completer analysis for the primary endpoints will be repeated for the key secondary endpoints. Subgroup analysis will be repeated for proportion of patients with NASH resolution and fibrosis improvement ≥1 stage at Week 24 compared to baseline.

MRI-PDFF will be analyzed based on MRI-PDFF Analysis Set. All other secondary efficacy analyses will be analyzed based on the Full Analysis Set with confirmed fibrosis stage F2 or F3 and NAS ≥4 at baseline per 3-panel consensus read. For hepatic fat fraction by MRI-PDFF, ALT, Pro-C3, adiponectin, serum triglycerides, HDL-c, Non-HDL-c, LDL-c and HbA1c, absolute values, changes, and percentage changes at baseline and each scheduled post-baseline visits will be summarized. Change and percentage change from baseline to post-baseline visits

will be analyzed by a mixed model repeated measures (MMRM) method (use ANCOVA for single post-baseline measurement) with treatment group, visit week, and treatment-by-week interactions as main effects, baseline measurement and two stratification factors as covariates in the model. An unstructured covariance matrix will be used to represent the correlation of the repeated measures within each subject whenever the model converges. The model will provide the least square (LS) mean, SE and 2-sided 95% CI for mean change or percent change from baseline within and between treatments. P-values will be calculated to compare the treatment effect in each pegozafermin group to that in the placebo group at specific study weeks. Each treatment group comparison will be performed at a 2-sided 0.05 significance level.

Changes and percentage changes from baseline to post-baseline visit will be visualized via line plots.

If the mixed or ANCOVA model assumption of the MMRM method is severely violated, the non-parametric van Elteren test stratified by T2DM status (yes vs. no) and fibrosis stage (F2 vs. F3) will be performed. The location shift estimate, and Hodges-Lehmann 2-tailed 95% confidence interval will be presented. For the comparison between the individual dose group and the placebo, if small cell counts (<5) render the van Elteren test inappropriate, the un-stratified Wilcoxon rank-sum test will be used instead.

7.8.3. Exploratory Efficacy Analysis

All other exploratory efficacy endpoints listed in Section 4.1.6 will be analyzed based on Full Analysis Set with confirmed fibrosis stage F2 or F3 and NAS ≥4 at baseline per 3-panel consensus read unless otherwise specified. Subject FAST score defined below will also be analyzed.

$$FAST = \{\exp(-1.65 + 1.07 \times \ln(LSM) + 2.66 \times 10^{-8} \times CAP^3 - 63.3 \times AST^{-1})\}/\{1 + \exp(-1.65 + 1.07 \times \ln(LSM) + 2.66 \times 10^{-8} \times CAP^3 - 63.3 \times AST^{-1})\}$$

For all proportion endpoints, the same stratified CMH methodology as the analysis of primary efficacy endpoints (described in Section Section 7.8.1) will be used to compare the differences of responder rate in each pegozafermin group versus placebo.

For change form baseline and percent change from baseline endpoints listed Section 4.1.6, descriptive summaries of absolute value and change from baseline at Week 24 will be provided. If there is only a single scheduled post-baseline assessment in Main Study, change from baseline will be analyzed by analysis of variance (ANCOVA) with treatment group, baseline measurement, and two stratification factors as covariates. Otherwise, MMRM method will be used as described in Section 7.8.2. Same as secondary efficacy endpoints, non-parametric van Elteren test stratified by T2DM status (yes vs. no) and fibrosis stage (F2 vs. F3) will be performed if the MMRM (ANCOVA) model assumptions are severely violated. The location shift estimate, and Hodges-Lehmann 2-tailed 95% confidence interval will be presented. For the comparison between each pegozafermin group and placebo, if small cell counts (<5) render the van Elteren test inappropriate, the un-stratified Wilcoxon rank-sum test will be used instead.

7.9. Patient Report Outcome (PRO) Analysis

All PRO summaries will be provided based on the observed data for the Full Analysis Set with confirmed fibrosis stage F2 or F3 and NAS ≥4 at baseline per 3-panel consensus read.

Chronic Liver Disease Questionnaire - NAFLD-NASH (CLDQ NAFLD-NASH)

CLDQ NAFLD-NASH contains 36 items (questions) grouped into 6 HRQL domains:

- Abdominal symptoms
- Activity
- Emotional
- Fatigue
- Systemic symptoms
- Worry

Each item's response ranges from 1 to 7. The score of each domain is calculated as the average of the domain's items (sum of all item's response divided by the number of non-missing items). The total CLDQ-NAFLD score will be calculated as an average of the six domain scores.

No imputation will be done for missing value. Surveys with more than 4 missing item responses will be excluded. If there is more than one missing item in one domain, missing value will be assigned for that domain. Total score will be set to missing if any domain score is missing.

CLDQ NAFLD-NASH summary statistics along with the change from baseline will be provided for each domain and total score, based on the full analysis set.

7.9.1. Work Productivity and Activity Impairment Questionnaire for NASH (WPAI-NASH)

WPAI-NASH includes 4 scores on a scale of 0 to 100%: absenteeism (the percentage of work time missed because of one's health), presenteeism (the percentage of impairment experienced while at work because of one's health), overall work impairment (a combination of absenteeism and presenteeism) and activity impairment (the percentage of impairment in daily activities because of one's health).

Summary statistics will be provided for absenteeism, presenteeism, overall work impairment, and activity impairment respectively.

7.9.2. Europe Quality of Life Group 5 Dimension 5 Level Questionnaire (EQ-5D-5L)

The EQ-5D-5L consists EQ-5D descriptive system and the EQ VAS.

EQ-5D descriptive system comprises five dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression). Each dimension has 5 levels (no problems, slight problems, moderate problems, severe problems, and extreme problems) with scale 1 to 5. Each domain will be summarized categorically (1-5).

EQ VAS score ranges from 0 (worst imaginable health) to 100 (best imaginable health). Descriptive summary statistics for the EQ VAS scores and the change from baseline will be provided.

7.9.3. The Appetite Sensations Visual Analog Scale (VAS)

In this study, VAS measures hunger; satiety; fullness; prospective food consumption; and desire to eat something fatty, salty, sweet, or savory, on a scale of 0 to 100.

Summary statistics will be provided for each domain.

7.9.4. Liver-related Pain and Discomfort Questionnaire

Liver-related Pain and Discomfort Questionnaire data will be listed.

7.10. Safety Analysis for Main Study

All safety analyses of Main Study will be performed on the Safety Analysis Set. Safety data presented by treatment group will be summarized on an 'as treated' basis. Safety variables include TEAEs, clinical laboratory parameters, ECG, vital signs, physical examination, bone mase density, and immunogenicity.

7.10.1. Adverse Events

Subject incidence of TEAEs will be tabulated by MedDRA (Version 24.0) system organ class, MedDRA preferred term, and treatment groups. All TEAEs, all treatment-emergent related AEs, all TESAEs, and all treatment-emergent serious related AEs, the most severe Common Terminology Criteria for Adverse Events (CTCAE) grade, TEAEs leading to dose interruption, TEAEs leading to IP discontinuation, and TESAEs leading to IP discontinuation will be summarized. Subjects will be counted only once within a SOC and Preferred Term (PT), even if the subject experienced more than one TEAE within a specific SOC and PT, and PT only.

Selected TEAE analyses will be repeated in subgroups including age, sex, race, and BMI.

All AEs, SAEs, AEs leading to IP interruption or discontinuation, fatal AEs, and COVID-19 related AEs will be listed with TEAE flags included.

Clinical Laboratory Parameters

Clinical laboratory tests listed in (Clinical Laboratory Tests) will be collected during the study.

For all other assessments that are below the lower limit of quantification will be imputed to half of the lower limit of quantitation (LLOQ) for summarization; assessments that are above the upper limit of quantification will be imputed to the upper limit of quantitation (ULOQ) for summarization. For listings, records that are LLOQ or ULOQ will be listed as such together with the imputed value in separate columns.

For all hematology, biochemistry urinalysis tests, coagulation factors, lipid assessments, and selected other laboratory assessments, descriptive summaries of absolute values and change from baseline at each scheduled assessment will be produced by treatment groups, the number and percentage of subjects with abnormal laboratory values will be summarized.

Shifts (low/normal/high) from the relevant baseline tables based on the normal ranges will be constructed. The shifts will be performed from the baseline to each post-baseline visit, and to minimum and maximum post-baseline visit.

A summary of suspected liver injury cases according to drug induced liver injury (DILI) criteria will also be presented (Table 6).

Table 6: Suspected liver injury cases according to drug induced liver injury (DILI) criteria

Baseline Value of ALT/AST	Criteria to discontinue investigational product
>ULN and <2× ULN	ALT or AST >5× baseline value
>=2× ULN but <5× ULN	ALT or AST >3× baseline value
>=5× ULN	ALT or AST >2× baseline value
>ULN	ALT or AST >2× baseline value and (BILI >2× ULN or INR >0.2)
Within Normal Range	ALT or AST >8× ULN
	ALT or AST >5× ULN
	ALT or AST >3× ULN and (BILI >2× ULN or INR >1.5)

7.10.2. Vital Signs

The observed values and the change from baseline for vital signs (body temperature, pulse rate, respiratory rate, and supine blood pressure) will be summarized by visit and treatment group.

The number and percent of subjects meeting the following criteria will be summarized considering all post-baseline visits including unscheduled:

- Absolute value of Systolic blood pressure (SBP) < 90 mm Hg
- Absolute value of Diastolic blood pressure (DBP) < 50 mm Hg
- Pulse rate <50 bpm
- Pulse rate > 120 bpm
- Maximum increase from baseline in SBP ≥30 mm Hg
- Maximum increase from baseline in DBP ≥20 mm Hg
- Maximum decrease from baseline in SBP ≥30 mm Hg
- Maximum decrease from baseline in DBP ≥20 mm Hg.

Clinically significant shifts for blood pressure from baseline to each post baseline visit will be summarized in the following criteria:

Table 7: Blood Pressure Category

Systolic (mm Hg)		Diastolic (mm Hg)	Blood Pressure Category
>180	Or	>120	Crisis
≥140	Or	≥90	Hypertension Stage 2
130-139	Or	80-89	Hypertension Stage 1
120-129	And	<80	Elevated
<90	Or	<60	Hypotension
<120	And	<80	Normal

7.10.3. Physical Examinations

Physical examination (abdominal, cardiovascular, neurological, respiratory, skin, and general, etc.) results will be summarized by visits and by treatment group.

7.10.4. Electrocardiograms

The following quantitative ECG measurements will be taken during the study:

- heart rate (bpm)
- PR interval (msec)
- Complex in ECG representing ventricular depolarization (QRS) interval (msec)
- Fridericia corrected QT (QTcF) interval (msec)

Descriptive summaries and changes from baseline will be presented by treatment group for the quantitative ECG measurements listed above and the qualitative overall ECG interpretation (categorized as normal; abnormal, insignificant; and abnormal, significant).

Shift tables will present changes from baseline in ECG interpretation (categorized as normal; abnormal, not clinically significant; and abnormal, clinically significant) to each post-baseline visit.

QTcF outliers (value >450 msec or change from baseline >30 msec) will summarized and flagged in listing.

7.10.5. Bone Mineral Density

Descriptive summaries of absolute value, change and percent change from baseline at each post-baseline assessment in BMD will be presented by treatment group using DXA results.

7.10.6. Immunogenicity

The number and percentage of subjects who develop anti-pegozafermin antibodies will be summarized at each visit by treatment group and pooled pegozafermin group. For those with a positive assessment, the ADA titers will also be summarized. The number and percentage of ADA positive samples with binding specificity (PEG domain specificity and FGF21 domain specificity) and neutralizing immunogenicity will also be summarized. Additional summary tables may be requested.

7.10.7. Cortisol Assessments

Cortisol assessment is performed at screening to obtain baseline values and at subsequent visits to assess for hypercortisolism while on treatment. The proportion of subjects with ≥ 15 nmol/L change from baseline of NSC (Night-time salivary cortisol) will be summarized by treatment and placebo group.

7.11. Serum concentration of pegozafermin

The serum concentration of pegozafermin over time will be listed by individual and summarized for each timepoint by treatment group. Serum concentration data will also be presented using arithmetic statistics. Individual and mean pegozafermin serum concentration vs. time plots will be produced on both a linear scale and on a semi-logarithmic scale.

For summarization of PK concentration values and data analysis, assessments that are below the lower limit of quantification (LLOQ) will be set to zero prior to summarization. For PK concentration graphs using a semi-logarithmic scale, assessments that are below the lower limit

of quantification will be set to half of LLOQ prior to graphing for illustration purposes. For PK concentration assessments that are above the upper limit of quantification (ULOQ), the record will be set to missing prior to summarization or graphing.

7.12. Final Analysis

All safety and efficacy tables in final analysis will be presented as:-

Description	Placebo Only Pooled	Pegozafermin 15 mg QW	Pegozafermin 30 mg QW Only	Pegozafermin 44 mg Q2W	Placebo (main) and Pegozafermin 30 mg QW (Extension)

Groups "Placebo Only Pooled" and "Pegozafermin 30 mg QW Only" contain subjects who received the same treatment during the Main Study and the Extension Study. Group "Placebo (main) and Pegozafermin 30 mg QW (Extension)" contains subjects who switched to Pegozafermin 30 mg QW treatment in the Extension Study.

Safety summaries for final analysis will be presented by the actual treatment subject received based on the Safety Analysis Set except for subjects who re-randomized to Pegozafermin 30mg treatment group in the Extension Study. Subjects who switched the treatment group at Extension Study will be summarized under "Placebo (main) and Pegozafermin 30 mg QW (Extension)" group.

Final Efficacy analysis (secondary endpoints of Extension Study) will be performed based on the Full Analysis Set with confirmed fibrosis stage F2 or F3 and NAS ≥4 at baseline per 3- panel consensus read and by the randomized treatment group in Main Study.

7.12.1. Primary endpoint - Adverse Events

All AE summaries in primary analysis for the Main Study (described in Section 7.10.1) will be repeated for in final analysis based on safety analysis set, extend to the pooled data of both Main Study and Extension Study.

7.12.2. Secondary Endpoints Analysis for Extension Study

Week 48 absolute change and percent change from baseline for each Pegozafermin individual dose level group, and for group who re-randomized to Pegozafermin 30mg in the Extension Study will be compared to "Placebo Only Pooled" group using the MMRM method with treatment group, week, and treatment-by-week interactions as main effects, baseline measurement and two stratification factors (T2DM status (yes vs. no) and fibrosis stage (F2 vs. F3)) as covariates in the model. The following clinical parameters will be analyzed:

- Hepatic fat fraction by MRI-PDFF
- ALT
- Pro-C3

An unstructured covariance matrix will be used to represent the correlation of the repeated measures within each subject whenever the model converges.

7.12.3. Safety, PK, and Exploratory Endpoints for Extension Study

Change from baseline and percentage change from baseline at each post baseline scheduled visits for all safety and exploratory endpoints listed in Section 4.2.3 and Section 4.2.4 will be summarized at final analysis based on safety analysis set, following the same layout as the primary analysis but extend to the pooled data of both Main Study and Extension Study.

The proportion of patients with ≥17 U/L decrease in ALT at each visit of Main Study and Extension Study will be summarized.

PK analysis will be repeated in final analysis including PK data from both Main Study and Extension Study. Completed Immunogenicity data including data collected during the immunogenicity follow-up period will be analyzed separately.

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APPENDICES

APPENDIX 1. SCHEDULE OF ACTIVITIES (SOA)

Subjects will attend clinic visits for Screening, Baseline (Day 1), Week 4, Week 12, Week 24, Week 38 and Week 48/ET. Note the sections referred in the table correspond to the sections in the protocol.

Assessments	**Screening Period**[1]	**Treatment Period**										**FU Period**		**Notes:** 1. Individual assessments may be done remotely as part of tiered screening. Screening period may be extended with Sponsor's approval 2. Clinic visit only (Baseline; Randomization Day) 3. If a required clinic visit is not possible, contact the Medical Monitor (or designee) and refer to Section 10.6 (Appendix 6). 4. May be a remote (home) visit 5. May be a remote (home) visit for IP administration 6. ET assessments to be performed for subjects who withdraw from study prematurely 7. Site staff to contact subject to assess for AEs and concomitant medications
		Main Study						**Extension Study**						
Study Day		**1**[2]												
Study Weeks	**-12 to 0**		**4**[3]	**8**[4]	**12**[3]	**16**[4]	**24**[3]	**26**[5]	**30**[4]	**38**[3]	**48**[3]	**ET**[3,6]	**51**[7]**/ EOS**	
Visit window (days)			±2	±7	±7	±7	+7	+7	±7	±14	+14		±14	
Informed consent	X													
Medical history/ demographics	X													
Liver biopsy	X [a]						X[b]					X[c]		Liver biopsies should ideally be done after MRI-PDFF. **a.** Must meet study inclusion criteria. A biopsy performed within 6 months of screening is acceptable instead of the baseline liver biopsy if the sample is deemed interpretable and eligible by the central reader. **b.** To be performed at -7 to +14 days of the Week 24 target visit; if biopsy not performed by Week 26 for subjects in QW groups, W26 dose to be held until discussion with Sponsor **c.** Applicable for subjects who discontinue IP at or after Week 16 and before Week 24 of the Main Study.
Histology machine read (PathAI)	X						X					X[c]		**c.** Applicable for subjects who discontinue IP at or after Week 16 and before Week 24 of the Main Study.
Prior medications	X													
Inclusion and exclusion criteria	X	B												

Assessments	**Screening Period**[1]	**Treatment Period**										**FU Period**		**Notes:** 1. Individual assessments may be done remotely as part of tiered screening. Screening period may be extended with Sponsor's approval 2. Clinic visit only (Baseline; Randomization Day) 3. If a required clinic visit is not possible, contact the Medical Monitor (or designee) and refer to Section 10.6 (Appendix 6). 4. May be a remote (home) visit 5. May be a remote (home) visit for IP administration 6. ET assessments to be performed for subjects who withdraw from study prematurely 7. Site staff to contact subject to assess for AEs and concomitant medications
		Main Study						**Extension Study**						
Study Day		**1**[2]												
Study Weeks	**-12 to 0**		**4**[3]	**8**[4]	**12**[3]	**16**[4]	**24**[3]	**26**[5]	**30**[4]	**38**[3]	**48**[3]	**ET**[3,6]	**51**[7]/ **EOS**	
Visit window (days)			±2	±7	±7	±7	+7	+7	±7	±14	+14		±14	
Complete physical exam	X[d]						X				X			**d.** includes recording height, weight, and calculating BMI.
Limited physical assessment[e]		B	X	X	X	X			X	X		X		**e.** if clinically indicated. May be performed by home health nurse for remote visits.
Body weight	X	B			X		X			X	X	X		The Investigator will be requested to further evaluate subjects with weight loss of ≥15.0% at Week 12 and ≥20.0% at Week 24, from baseline, for the potential cause of weight loss.
Waist and hip circumferenc	X	X			X		X			X	X	X		
Lifestyle counseling	X	X	X	X	X	X	X		X	X				Strenuous exercises should be avoided for at least 48 hours prior to study visits.
Fibroscan VTCE and CAP	X[f]						X				X	X		**f.** Fibroscan should ideally be performed prior to MRI-PDFF. At Screening, a historical Fibroscan assessment performed within the last 3 months prior to screening may be acceptable.
12-lead ECG (single)[g]	X	B			X		X			X	X	X		**g.** ECGs will be recorded as single bedside measurements. Additional ECG may be conducted if clinically indicated. ECG should be performed prior to vital signs and laboratory assessments. All ECG data should be provided within 72 hours to the central reader for assessment.
Urine drug screen	X[h]													**h**. Urine drug screen will be done locally with a standardized kit during screening period by Day 1. If cannot be performed during screening, test can be performed on Day 1, but must be completed before randomization procedures start. Refer to Appendix 2 (Section 10.2).

Assessments	**Screening Period**[1]	**Treatment Period**										**FU Period**		**Notes:** 1. Individual assessments may be done remotely as part of tiered screening. Screening period may be extended with Sponsor's approval 2. Clinic visit only (Baseline; Randomization Day) 3. If a required clinic visit is not possible, contact the Medical Monitor (or designee) and refer to Section 10.6 (Appendix 6). 4. May be a remote (home) visit 5. May be a remote (home) visit for IP administration 6. ET assessments to be performed for subjects who withdraw from study prematurely 7. Site staff to contact subject to assess for AEs and concomitant medications
		Main Study						**Extension Study**						
Study Day		**1**[2]												
Study Weeks	**-12 to 0**		**4**[3]	**8**[4]	**12**[3]	**16**[4]	**24**[3]	**26**[5]	**30**[4]	**38**[3]	**48**[3]	**ET**[3,6]	**51**[7]**/ EOS**	
Visit window (days)			±2	±7	±7	±7	+7	+7	±7	±14	+14		±14	
Hematology, and coagulation	X	B	X		X		X		X	X	X	X		Refer to Appendix 2 (Section 10.2) for laboratory parameters.
Biochemistry	X[i]	B	X	X	X		X		X	X	X	X		Refer to Appendix 2 (Section 10.2) for laboratory parameters. **i.** For all subjects, ALT and AST will be collected twice during the Screening period at least 2 weeks apart between the 1st and 2nd assessment. A 3rd assessment, if required, will be collected via unscheduled visit, and performed at least 1 week apart from the 2nd assessment (refer to Exclusion criterion in Section 5.2.)
FSH	X													Only if required to confirm menopausal status in women under the age of 45 who have amenorrhea >12 months and are not using hormonal contraception or hormone replacement therapy.
LH, FSH, Estradiol		X					X				X			Only for WOCBP who are not on hormonal contraception.
Urinalysis	X	B	X		X		X			X	X	X		
HbA1c	X	B			X		X			X	X	X		
HOMA-IR	X	B			X		X			X	X	X		
IGF-1		B			X		X			X	X	X		
Serology	X													Refer to Appendix 2 (Section 10.2) for laboratory parameters (HBsAg, HCV and HIV 1 and 2 antibodies [HCV RNA reflex for HCV Ab+ only]).
Thyroid panel (TSH, FT4, TT3)	X				X		X			X	X	X		

Assessments	**Screening Period**[1]	**Treatment Period**										**FU Period**		**Notes:** 1. Individual assessments may be done remotely as part of tiered screening. Screening period may be extended with Sponsor's approval 2. Clinic visit only (Baseline; Randomization Day) 3. If a required clinic visit is not possible, contact the Medical Monitor (or designee) and refer to Section 10.6 (Appendix 6). 4. May be a remote (home) visit 5. May be a remote (home) visit for IP administration 6. ET assessments to be performed for subjects who withdraw from study prematurely 7. Site staff to contact subject to assess for AEs and concomitant medications
		Main Study						**Extension Study**						
Study Day		**1**[2]												
Study Weeks	**-12 to 0**		**4**[3]	**8**[4]	**12**[3]	**16**[4]	**24**[3]	**26**[5]	**30**[4]	**38**[3]	**48**[3]	**ET**[3,6]	**51**[7]/ **EOS**	
Visit window (days)			±2	±7	±7	±7	+7	+7	±7	±14	+14		±14	
Pregnancy test in WOCBP only[j]	X (Serum)	B	X	X	X	X	X		X	X	X	X		**j.** Serum pregnancy test will be conducted at Screening; at all other timepoints urine pregnancy test will be done. For in-clinic visits at the indicated timepoints, a urine pregnancy test will be obtained locally. If the urine test is positive, dosing should be withheld, a confirmatory serum pregnancy test should be sent to the central laboratory, and the Sponsor should be notified.
Vital signs[k] (blood pressure, pulse, body temperature, and respiratory rate)	X	B	X	X	X	X	X		X	X	X	X		**k.** Vital signs will be measured prior to scheduled blood draws. Starting from randomization, blood pressure and pulse will be measured in duplicate, the first measurement will be taken up to 15 minutes before the indicated timepoint. Additional vital signs measurement may be done if clinically indicated. Subjects must be in a supine or semi-erect/seated position and resting for at least 5 minutes prior to measurements. Repeat measurements should be performed according to local practice.
Patient reported		B			B		B				X	X		
Randomization		X					X[l]							**l. for Extension Study (Placebo QW)** At Week 24, subjects randomized to placebo QW in the Main Study will be re-randomized 1:1 to pegozafermin 30 mg QW (n=21) or placebo QW (n=21).

Assessments	**Screening Period**[1]	**Treatment Period**										**FU Period**		**Notes:** 1. Individual assessments may be done remotely as part of tiered screening. Screening period may be extended with Sponsor's approval 2. Clinic visit only (Baseline; Randomization Day) 3. If a required clinic visit is not possible, contact the Medical Monitor (or designee) and refer to Section 10.6 (Appendix 6). 4. May be a remote (home) visit 5. May be a remote (home) visit for IP administration 6. ET assessments to be performed for subjects who withdraw from study prematurely 7. Site staff to contact subject to assess for AEs and concomitant medications
		Main Study						**Extension Study**						
Study Day		**1**[2]												
Study Weeks	**-12 to 0**		**4**[3]	**8**[4]	**12**[3]	**16**[4]	**24**[3]	**26**[5]	**30**[4]	**38**[3]	**48**[3]	**ET**[3,6]	**51**[7]**/ EOS**	
Visit window (days)			±2	±7	±7	±7	+7	+7	±7	±14	+14		±14	
Dispense IP		X	<=========X[m] ==========>											**m.** Sponsor-approved courier services may provide IP to subjects from Week 4 through EOS. Depending on geography this may be direct-to-subject shipment or may require site to-subject shipment. Instructions for dispensation and IP shipment to site and direct-to-subject shipment will be detailed in the Pharmacy Manual. IP should be dispensed approximately every 4 weeks.
IP administration[n]		X	QW from Weeks 1 to 23				QW from Weeks 24[o] to 47							Dosing window is ±2 days, however for QW dosing, every effort should be made to take IP on the same day of the week and must be at least 5 days between 2 doses. **n.** IP will be administered SC to the abdomen by trained and qualified study personnel, site staff at clinic visit(s), or at home by healthcare professional, or subject's caregiver for home administration. IP may also be self-administered at the subject's home.
			Q2W from Weeks 2 to 22				Q2W from Weeks 24 to 46							
														o. Week 26 may be an in-clinic or a remote/home visit by healthcare professional who will administer the IP.
PK blood collection[p]		B	B	B	B		B			B	X	X		**p.** PK blood samples will be collected predose. *Additional blood samples for PK analysis may be collected if clinically indicated (e.g., in case of SAE)*. For PK sample collection instruction/ procedures, refer to the relevant manual.

Assessments	**Screening Period**[1]	**Treatment Period**										**FU Period**		**Notes:** 1. Individual assessments may be done remotely as part of tiered screening. Screening period may be extended with Sponsor's approval 2. Clinic visit only (Baseline; Randomization Day) 3. If a required clinic visit is not possible, contact the Medical Monitor (or designee) and refer to Section 10.6 (Appendix 6). 4. May be a remote (home) visit 5. May be a remote (home) visit for IP administration 6. ET assessments to be performed for subjects who withdraw from study prematurely 7. Site staff to contact subject to assess for AEs and concomitant medications
		Main Study						**Extension Study**						
Study Day		**1**[2]												
Study Weeks	**-12 to 0**		**4**[3]	**8**[4]	**12**[3]	**16**[4]	**24**[3]	**26**[5]	**30**[4]	**38**[3]	**48**[3]	**ET**[3,6]	**51**[7]**/ EOS**	
Visit window (days)			±2	±7	±7	±7	+7	+7	±7	±14	+14		±14	
ELF panel, Pro-C3, adiponectin, FIB-4 index (derived calculation), and adipo-IR (fasting free fatty acids x fasting insulin)		B			X		X			X	X	X		Refer to Appendix 2 (Section 10.2).
Night-time salivary cortisol (NSC)[q]	X[q]				X		X			X	X	X		**q.** NSC sample will be collected by subject at home between 8:00pm and 12:00am before submitting the sample for processing at the specified visit. If the sample is not collected between 8:00pm and 12:00am, the test should be repeated. The screening assessment should be performed as early as possible during the screening period. Subjects should be provided with NSC collection kit at the first screening visit, when possible, to allow sample collection return at the following clinic visit. If screening value is >15 nmol/L, Medical Monitor (or designee) may request further workup should the value be of clinical concern; however, values >15 nmol/L will not be exclusionary. The screening NSC is a safety assessment, and not an I/E criteria. Refer to Section 10.2 for further requirement for Value >15 nmol/L on treatme

Assessments	**Screening Period**[1]	**Treatment Period**										**FU Period**		**Notes:** 1. Individual assessments may be done remotely as part of tiered screening. Screening period may be extended with Sponsor's approval 2. Clinic visit only (Baseline; Randomization Day) 3. If a required clinic visit is not possible, contact the Medical Monitor (or designee) and refer to Section 10.6 (Appendix 6). 4. May be a remote (home) visit 5. May be a remote (home) visit for IP administration 6. ET assessments to be performed for subjects who withdraw from study prematurely 7. Site staff to contact subject to assess for AEs and concomitant medications
		Main Study						**Extension Study**						
Study Day		**1**[2]												
Study Weeks	**-12 to 0**		**4**[3]	**8**[4]	**12**[3]	**16**[4]	**24**[3]	**26**[5]	**30**[4]	**38**[3]	**48**[3]	**ET**[3,6]	**51**[7]/ **EOS**	
Visit window (days)			±2	±7	±7	±7	+7	+7	±7	±14	+14		±14	
MRI-PDFF	X				X		X				X	X		At Screening, MRI-PDFF should ideally be done before liver biopsy. At Screening, a historical MRI-PDFF assessment performed within the last 3 months prior to screening may be acceptable if the images are available and evaluable by the central imaging vendor. On treatment, MRI-PDFF should ideally be done within ± 7 days of the target visit date. If out of the window, MRI-PDFF should be performed as close to the target visit date.
cT1	X				X		X				X	X		Iron-corrected T1 mapping. Applicable only at sites capable of conducting cT1 imaging. At Screening, a historical cT1 assessment performed within the last 3 months prior to screening may be acceptable if the images are available and evaluable by the central imaging vendor.
Liver/Spleen volume and pancreatic fat	X				X		X				X	X		To be done at the same time as MRI-PDFF. At Screening, a historical liver/spleen volume and pancreatic fat assessment performed within the last 3 months prior to screening may be acceptable if the images are available and evaluable by the central imaging vendor.
Bone Mineral Density (BMD) by Dual X Ray Absorptiometry (DXA)[r]		X					X				X	X		**r.** DXA should be done within -14/+7 days of the target visit date. If out of the window, DXA should be performed as close to the target day as possible.
Plasma for bone biomarkers		B			X		X				X	X		Includes CTX, P1NP, and osteocalcin; refer to Appendix 2 (Section 10.2).

Assessments	**Screening Period**[1]	**Treatment Period**										**FU Period**		**Notes:** 1. Individual assessments may be done remotely as part of tiered screening. Screening period may be extended with Sponsor's approval 2. Clinic visit only (Baseline; Randomization Day) 3. If a required clinic visit is not possible, contact the Medical Monitor (or designee) and refer to Section 10.6 (Appendix 6). 4. May be a remote (home) visit 5. May be a remote (home) visit for IP administration 6. ET assessments to be performed for subjects who withdraw from study prematurely 7. Site staff to contact subject to assess for AEs and concomitant medications
		Main Study						**Extension Study**						
Study Day		**1**[2]												
Study Weeks	**-12 to 0**		**4**[3]	**8**[4]	**12**[3]	**16**[4]	**24**[3]	**26**[5]	**30**[4]	**38**[3]	**48**[3]	**ET**[3,6]	**51**[7]**/ EOS**	
Visit window (days)			±2	±7	±7	±7	+7	+7	±7	±14	+14		±14	
Endogenous FGF21[s]		B												**s.** Baseline samples of endogenous FGF21 will be obtained in all subjects; additional samples will be obtained at the immunogenicity follow-up visits (See the SOA for immunogenicity follow-up period). Endogenous FGF21 samples (baseline and follow-up visit) will be analyzed only in
Immunogenicity		B	X	X	X		X			X	X[t]	X		See the SOA for immunogenicity follow-up.
Pharmacogenomic (DNA) blood sampling		X												Optional.
Blood sample for exploratory biomarkers		X					X				X	X		
Adverse event monitoring[u]	X	X	<==========X ==========>									X	X	**u.** The sites may take non-personally identifying photographs of potential injection site reactions (optional).
Concomitant	X	X	<==========X ==========>									X	X	

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; B, predose; CTX, carboxy-terminal collagen crosslinks; D, Day; ECG=electrocardiogram; ELF, enhanced liver fibrosis; EOS, end of study; ET= early termination; FGF21, fibroblast growth factor 21; FIB-4, Fibrosis-4 index; FU, Follow-Up; HbA1c= glycated hemoglobin; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HOMA- IR, homeostatic model assessment for insulin resistance; I/E, inclusion/exclusion; IGF-1, insulin-like growth factor 1; IP, investigational product; LH, luteinizing

hormone; MRI-PDFF, magnetic resonance imaging based proton density fat fraction; NSC, night-time salivary cortisol; P1NP, N- terminal propeptide of type 1 collagen; PK=pharmacokinetic; Pro-C3, N-terminal propeptide of type III collagen; QW, once weekly; Q2W, every 2 weeks; S, serum; TSH, thyroid stimulating hormone; WOCBP=women of child-bearing potential

Schedule of Activities (Immunogenicity Follow-up Period)

Assessments	**Immunogenicity Follow-up Period**				**Notes:**
Visit	**FU Visit 1**	**FU Visit 2**	**FU Visit 3**	**FU Visit 4**	**1.** Informed consent (addendum) for the immunogenicity follow-up period will be performed at the subject's next scheduled visit from the effective date of Protocol Amendment 3 informed consent. For subjects who have completed the study and ≥ 3 months have elapsed since last dose, informed consent should be obtained as soon as possible. 2. Subjects who terminate IP early will not return for immunogenicity follow-up, unless asked to return for additional testing(s), at the Medical Monitor's discretion.
Study Week	**60**	**72**	**84**	**96**	
Visit Window (Days)	± 14	± 14	± 14	± 14	
Immunogenicity[a]	X	X	X	X	**a.** Subjects who complete the Week 48 visit will be asked to return to clinic to have blood samples collected approximately every 3 months for up to one year. Additional testing may be requested in the event of safety-related concerns. The sponsor may elect to terminate participation in the immunogenicity follow-up period based on emerging data.
Endogenous FGF21[b]		X		X	**b.** Endogenous FGF21 samples will only be analyzed for NAb+ subjects.
Adverse event monitoring[c]	X	X	X	X	**c.** Immunogenicity testing for a longer follow-up may be requested in the event of safety-related concerns.
Concomitant medications[d]	X	X	X	X	**d**. Changes and new concomitant medication will be recorded.

Abbreviations: FGF21 = fibroblast growth factor 21; FU = follow-up; Nab+ = Neutralizing antibody positive

APPENDIX 2. MAIN STUDY EXPLORATORY PROPORTION ENDPOINTS

Table 8: Histological assessments Proportion endpoints

Proportion of subjects at Week 24 with:

- [1] NASH resolution without worsening of fibrosis and ≥ 2-point improvement in NAS score at Week 24 compared to baseline
- [2] Improvement of fibrosis ≥1 stage without worsening of NASH and ≥ 2-point improvement in NAS score at Week 24 compared to baseline
- [3] Fibrosis improvement ≥2 stage without worsening of NASH
- [4] NASH resolution without worsening of fibrosis, OR improvement of fibrosis ≥1 stage without worsening of NASH
- [5] At least a 2-point improvement in NAS with at least a 1-point improvement in ballooning or lobular inflammation, and no worsening of fibrosis
- [6] Decrease >1 stage in fibrosis score
- [7] At least 2-point improvement in NAS score AND are MRI-PDFF responders
- [8] Fibrosis improvement ≥1 stage without worsening of NASH AND are MRI-PDFF responders AND ALT responders
- [9] Fibrosis improvement ≥1 stage without worsening of NASH AND are MRI-PDFF responders
- [10] NASH resolution without worsening of fibrosis AND are MRI-PDFF responders AND ALT responders
- [11] NASH resolution without worsening of fibrosis AND are MRI-PDFF responders

In MRI-PDFF responders[8]:
Proportion of subjects with:

- [12] Fibrosis improvement ≥2 stage without worsening of NASH
- [13] NASH resolution without worsening of fibrosis, OR improvement of fibrosis ≥1 stage without worsening of NASH
- [14] NASH resolution AND fibrosis improvement ≥1 stage
- [15] At least a 2-point improvement in NAS with at least a 1-point improvement in ballooning or lobular inflammation, and AND no worsening of fibrosis
- [16] At least a 2-point improvement in NAS with at least a 1-point improvement in ballooning or lobular inflammation, and AND fibrosis improvement ≥1 stage

Table 9: Exploratory Proportion endpoints with each criterion

Criteria (at Week 24)	**[1]**	**[2]**	**[3]**	**[A]**	**[B]**	**[5]**	**[6]**	**[7]**	**[8]**	**[9]**	**[10]**	**[11]**	**[12]**	**[14]**	**[15]**	**[16]**
NASH resolution	Y			Y							Y	Y		Y		
No NASH worsening		Y	Y		Y				Y	Y			Y			
1-point improvement in ballooning or lobular inflammation						Y									Y	Y
No fibrosis worsening	Y			Y		Y					Y	Y			Y	
Fibrosis ≥ 1 stage improvement		Y			Y				Y	Y				Y		Y
Fibrosis ≥ 2 stage improvement			Y				Y						Y			
≥2 points NAS improvement	Y	Y				Y		Y							Y	Y
MRI-PDFF responder								Y	Y	Y	Y	Y	Y	Y	Y	Y
ALT responder									Y		Y					

NOTE: Exploratory End point [4] in Table 8 = criteria [A] OR [B]
Exploratory End point [13] in Table 8 = MRI-PDFF RESPONDER + (criteria [A] OR [B])

APPENDIX 3. CLINICAL LABORATORY TESTS

The clinical laboratory tests detailed in below will be performed by a central laboratory, except if noted otherwise, at timing/frequency detailed in the Appendix 1 SoA. Laboratory tests will be performed under fasting conditions (≥10 hours).

Hematology	
White blood cell count (WBC) with differential (Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils – absolute and %)	Red blood cell (RBC) RBC Indices: Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), %Reticulocytes
Hemoglobin	Hematocrit
Platelet count	Red cell Distribution Width
Coagulation factors: prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT)	
Biochemistry	
Alanine Aminotransferase (ALT)	Aspartate Aminotransferase (AST)
Alkaline phosphatase (ALP)	Gamma-glutamyl transferase (GGT)
Total bilirubin, Indirect/direct bilirubin	Albumin
Calcium	Blood urea nitrogen (BUN)
Sodium	Creatinine
Chloride	Creatine kinase
Magnesium	Bicarbonate
Potassium	Lactate dehydrogenase
Phosphorus/	Total protein
Glucose (fasting)	Total cholesterol
	Uric acid
Serum lipids: triglycerides, high-density lipoprotein cholesterol (HDL-c), low density lipoprotein (LDL-c), non-HDL-c	

<table>
<tr><th colspan="2">Urinalysis (spot urine)</th></tr>
<tr><td>Basic Urinalysis (dipstick, including macroscopic appearance, bilirubin, blood, color, glucose, ketones, leukocyte esterase, nitrite, pH, protein, specific gravity, urobilinogen);</td><td>Full urinalysis (dipstick plus microscopic evaluation) to be performed only at the Screening and Week 48/ET visits). A reflex microscopic urinalysis should be performed if the result of the urinalysis is abnormal or at the discretion of the Investigator or delegate.</td></tr>
<tr><th colspan="2">Other Study-Specific Laboratory Assessments</th></tr>
<tr><td>Pegozafermin (to be evaluated by bioanalytical laboratory)</td><td rowspan="2">Insulin
High-sensitivity C-reactive protein (hsCRP)
Hemoglobin A1c (HbA1c)
Insulin-like growth factor-1 (IGF-1), total
Adiponectin, total
C-peptide
Enhanced liver fibrosis (ELF) panel including concentrations of serum biomarkers: tissue inhibitor of matrix metalloproteinases-1 (TIMP-1), amino-terminal type 3 procollagen peptide (P3NP), and hyaluronic acid (HA)
N-terminal type 3 propeptide of collagen (Pro-C3)
Fasting free fatty acids (FFA) and Adipo-IR index (fasting FFA x fasting insulin)[a]
FIB-4 index (derived calculation)
Alpha feto-protein (AFP) (for F4 patients only)
MELD Na+ (for F4 patients only)</td></tr>
<tr><td>Serum and urine human chorionic gonadotropin (hCG) pregnancy test for women of childbearing potential
FSH (for confirmation of menopausal status in women under the age of 45 with >12 months amenorrhea not using hormonal contraception or hormone replacement therapy)
Luteinizing hormone (LH), FSH and estradiol (only for WOCBP who are not on hormonal contraception)
Thyroid panel: TSH, FT4 and TT3
Night-time salivary cortisol (NSC)*
* NSC samples must be collected between 8:00pm and 12:00am before submitting the sample for processing at the specified visit. If sample is not collected during this time, repeat sample must be collected. If screening value is >15 nmol/L, Medical Monitor (or designee) may request further workup should the value be of clinical concern; however, values > 15 nmol/L will not be exclusionary. The screening NSC is a safety assessment, and not an eligibility criteria. The screening assessment should be performed as early as possible during the screening period. Subjects should be provided with NSC collection kit at the first screening visit, when possible, to allow sample collection return at the following clinic visit.</td></tr>
</table>

For on-treatment values that are >15.0 nmol/L AND above the screening value, a repeat NSC should be performed (sample should be collected at approximately the same time as the screening sample). If this repeat assessment is still > 15.0 nmol/L AND above the screening value, an additional screening test for hypercortisolism will be done (either overnight dexamethasone (1 mg) suppression test or a 24 h collection for urinary free cortisol). Medical Monitor (or designee) will provide guidance on the appropriate assessment based on the individual subject for subsequent test values that are also >15 nmol/L based on the subject profile.	
Urine drug screen including amphetamines, barbiturates, cocaine metabolites, opiates, benzodiazepines and cannabinoids will be done at local laboratory using a standardized kit during screening period by Day 1. If cannot be performed during screening, test can be performed on Day 1, but must be completed before randomization procedures start. **Serology**: HIV 1 and 2 antibodies, hepatitis B surface antigen (HBsAg), hepatitis C virus antibody, and HCV RNA (only if HCV antibody-positive) **Immunogenicity**: Antibody to pegozafermin and NAb Endogenous FGF21	
Bone markers: carboxy-terminal collagen crosslinks (CTX), N-terminal propeptide of type 1 collagen (P1NP), osteocalcin Plasma and serum samples for exploratory biomarkers Biomarkers from RNA/DNA Pharmacogenomics sample	