CLINICAL STUDY PROTOCOL

TITLE PAGE

Title	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)
Short Title:	The ENLIVEN Study
Protocol Number:	BIO89-100-122
Compound Number:	BIO89-100 (Pegozafermin)
Study Phase:	Phase 2b
Sponsor Name:	89bio, Inc
Legal Registered Address:	142 Sansome Street, 2nd Floor San Francisco CA 94104, US
IND Number:	131934
Sponsor Representative:	[redacted]
Approval Date:	05 December 2022
Version:	Version 4.0
Amendment:	Amendment 3

This clinical study will be conducted in accordance with current Good Clinical Practice (GCP) as directed by the provisions of the International Council for Harmonisation (ICH) and with the ethical principles contained in the Declaration of Helsinki; United States (US) Code of Federal Regulations (CFR), and European Union (EU) Directives and Regulations (as applicable in the region of the study); national country legislation; and the Sponsor's Standard Operating Procedures (SOPs).

SIGNATURE PAGE

Sponsor Signatory:

05-Dec-2022

89bio, Inc

Date

Medical Monitor (or designee) Name and Contact Information will be provided separately.

PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Protocol Title	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)
Protocol Number	BIO89-100-122
Version and Date	Version 4.0, 05 December 2022
Amendment No.	3
IND Number	131934

I, the undersigned, have read this protocol and agree to personally supervise conduct of this protocol in accordance with ethical principles as outlined in the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice, any applicable laws and requirements (including Part 54: Financial Disclosure by Clinical Investigators) and any additional conditions mandated by a regulatory authority and/or Institutional Review Board/Independent Ethics Committee (IRB/IEC).

I acknowledge that I am responsible for the overall study conduct; I approve of and will comply with all conditions, instructions and restrictions described in this protocol. I am aware that my adherence to the above protocol is mandatory and that any changes in the protocol or consent form, except those necessary to eliminate apparent immediate hazards to human subjects, must first be approved in writing by 89bio, Inc and the respective IRB/IEC.

I also agree that all information provided to me by the Sponsor, including this document, Investigator's Brochure, case report form, and verbal and written information, will be kept strictly confidential and confined to the clinical personnel involved in conducting the study. It is recognized that this information may be related in confidence to the IRB/IEC. I also understand that reports of information about the study or its progress will not be provided to anyone not involved in the study other than to the Principal Investigator, or in confidence to the IRB/IEC or to the Food and Drug Administration (FDA) or other legally constituted authority.

Principal Investigator Signature	Date

Printed Name	Institution	City, Country

PROTOCOL AMENDMENT SUMMARY OF CHANGES

Document History

Document	Date
Version 1.0, Original	08 April 2021
Version 2.0, Amendment 1	01 December 2021
Version 3.0, Amendment 2	11 March 2022
Version 4.0, Amendment 3	05 December 2022

Summary of Changes in Protocol Amendment 3, Version 4.0:

Section Number and Heading	Description of Change	Brief Rationale
1.1 Synopsis and 1.2 Study Schema	All applicable changes made in the corresponding sections in the protocol were included in the synopsis and study schema.	See below for the rationale for specific changes.
1.3 Schedule of Activities (SoA)	• Added visit window of +7 days for the Week 26 visit. • Specified that all ECG data should be provided to the central reader within 72 hours, for assessment. • Clarified that IP should be dispensed ~ every 4 weeks and that 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. • Removed the requirement for Medical Monitor (designee) approval if MRI-PDFF and/or DXA assessments are performed out of window and widened the window for post-treatment DXA assessment. • Clarified the timepoint(s) at which endogenous FGF21 samples will be obtained.	• Clarifications for study procedures.
	• Increased the threshold for screening NSC that would require further workup, from > 6.0 to > 15 nmol/mL and clarified requirements for values >15 nmol/mL on treatment.	• To align NSC range to better monitor cortisol level.
	• Added Table 2 to describe the schedule of activities for the immunogenicity follow-up period of up to [redacted] with follow-up visits every 12 weeks after the Week 48 visit.	• Added immunogenicity follow-up period to evaluate the long-term immunogenicity effects of pegozafermin.
3.1 Main Study Objectives and Endpoints	• Revised the primary endpoints definition to the endpoints specified in the original protocol and consistent with the primary efficacy endpoints outlined in the FDA draft guidance on developing drugs for noncirrhotic NASH with liver fibrosis.	• To be consistent with the primary efficacy endpoints outlined in the FDA draft guidance.

Section Number and Heading	Description of Change	Brief Rationale
	• Moved the primary endpoints with the criterion of '≥2-point improvement in NAS score' to exploratory endpoints. • Clarified the safety endpoint definition for cortisol assessment and BMD assessment (main and extension study). • Removed the endpoint related to endogenous FGF21 in the Main study as there are no follow-up assessments during the main study. • Removed the redundant exploratory endpoint "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. • Added "percentage of subjects with ≥ 30% decrease in ALT" as an exploratory endpoint in both Main and Extension Study. • Clarified the endpoint related to FibroScan in the Main Study and added the same endpoint in Extension Study.	
4.1 Overall Study Design	• Included an immunogenicity follow-up period after the Week 48 visit, for immunogenicity assessment approximately every 3 months for up to [redacted].	• To evaluate the long-term immunogenicity effects of pegozafermin.
4.4 Study Duration	• The total duration of study participation was revised from ~64 weeks to ~ 112 weeks. • Defined the Immunogenicity follow-up period and specified the duration.	• To include the duration of the immunogenicity follow-up period.
4.5 End of Study Definition	• Clarified the definition of study completion and included the definition for end of immunogenicity follow-up period.	• Clarification and additional information.
5.2 Exclusion Criteria	• # 25: Specified that study participants will not be allowed to participate in other interventional trials for FGF21 analog or FGFR1 activating products, during the immunogenicity follow-up period.	• To avoid confounding factors in assessment of immunogenicity.
6.4.3 Blinding	• Clarified that *specifically-identified* Sponsor personnel will not be blinded and Sponsor personnel responsible for day-to-day study operations will remain blinded, including the Sponsor and CRO Medical Monitors. • Specified that a formal unblinding plan will be established prior to the unblinding for Week 24.	• Additional information.
6.5.1 Guidance for Missed Dose(s)	• Clarified instructions for QW dosing, that 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.	• Clarification.

Section Number and Heading	Description of Change	Brief Rationale
6.7.1 Prohibited Medications/Therapies	• Included elective bone surgery for bone disorders as prohibited therapy. • Added that TNF antagonists, adalimumab, etanercept, certolizumab, infliximab or sulfasalazine for rheumatoid arthritis are prohibited during the Main Study but may be considered during the Extension Study.	• For consistency with exclusionary criterion for bone surgeries.
	• Added that initiation of agents for uncontrolled hyperglycemia (but not weight loss) may be allowed during the Extension Study, with the Medical Monitor's approval.	• Additional information.
	• Specified that study participants will not be allowed to participate in other interventional trials for FGF21 analog or FGFR1 activating products, during the immunogenicity follow-up period.	• To avoid confounding factors in assessment of immunogenicity.
7.1 Discontinuation of IP and Subject withdrawal from the Study	• Provided additional reasons for discontinuation of IP or subject withdrawal from the study: (i) evidence of hepatic decompensation as assessed by investigator (ii) MELD Na+ score (for F4 subjects only).	• Added as safety measure for subjects with cirrhotic (F4) NASH.
	• Clarified the assessments to be performed for subjects who discontinue IP but do not withdraw from the study.	• Clarification.
7.1.2 Monitoring and Study Continuation of Subjects with NASH Fibrosis Stage F1 or F4 (new section)	• Provided further information on managing subjects in the study with NASH fibrosis stage F1 or F4.	• Added as safety measure for subjects with cirrhotic (F4) NASH. • Clarification that F1 will continue as an exploratory population.
8.2.5.1 Immunogenicity	• Revised to specify that 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 [redacted]	• To evaluate the long-term immunogenicity effects of pegozafermin.
	• Added that the samples for endogenous FGF21 level will be collected at the immunogenicity follow-up visit(s) and clarified that samples will be analyzed only for subjects [redacted].	• Clarification.
8.2.5.2 Cortisol Assessments	• Increased the threshold for screening NSC that would require further workup, from > 6.0 to > 15 nmol/mL and clarified requirements for values >15 nmol/mL on treatment.	• Changed threshold based on change in methodology and to relevant clinical levels for establishing hypercortisolism.
8.3.1 Time Period and Frequency for Collecting AE and SAE Information	• Added details on collection of AE and SAE information for the immunogenicity follow-up period.	• Additional information based on inclusion of the immunogenicity follow-up period.

Section Number and Heading	Description of Change	Brief Rationale
8.4 Pharmacokinetics	• Specified that PK samples collected from subjects during the period when they are administered placebo will not be analyzed.	• Clarification and additional information.
8.5.1 Pharmacodynamics	• Listed AFP and MELD Na+ score as parameters to be evaluated in F4 subjects only.	• Additional information.
9.1.1 Estimand Framework	• Specified the primary estimand of interest and methods of handling the intercurrent events defined for the study.	• Per ICH guidance for industry E9(R1) Statistical Principles for Clinical Trials: Addendum: Estimand and Sensitivity Analysis in Clinical Trials.
9.2 Sample Size Determination	• Revised the sample size justification and assumptions based on the revised primary endpoint definition.	• To update the sample size determination section based on the revised primary endpoints.
9.3 Population Analysis	• Defined the MRI-PDFF analysis set and removed the definition of extension analysis set. • Defined the randomized analysis set.	• To clarify analysis populations to be aligned with the study objectives.
9.4 Statistical Analyses	• Specified supportive analysis that will compare each QW and Q2W treatment arm to corresponding QW or Q2W placebo arm (not pooled).	• To add the supportive analysis to assess the robustness of the primary analysis results.
9.6 Primary Analysis	• Specified that the list of Sponsor personnel unblinded to the summary results will be recorded in a separate document.	• Additional information.
9.7 Final Analysis	• Clarified when the final analysis will be performed, as an immunogenicity follow-up period was added to the study.	• Clarification.
10.2 Clinical Laboratory Tests	• Clarified the collection and follow-up details for NSC. • Clarified the visits at which full urinalysis should be performed. • Included AFP assessment as a laboratory parameter for F4 patients only.	• Clarifications.
10.8 Child-Turcotte- Pugh Classification (new)	• Added the scoring parameters and interpretation for CTP score.	• Additional information for guidance.
Overall	Editorial and formatting changes, including updates to references.	To clarify and correct any errors or inconsistencies across sections.

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1. PROTOCOL SUMMARY

1.1. Synopsis

Protocol Title: 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)

Short Title: The ENLIVEN Study

Study Rationale

BIO89-100 (pegozafermin), developed by 89bio, is a glycoPEGylated analogue of fibroblast growth factor 21 (FGF21), an endogenous metabolic hormone secreted by the liver, adipose tissue, skeletal muscle, and the pancreas, that is regulated by nutritional status and affects energy expenditure and glucose and lipid metabolism (Arner, 2008; Lee, 2014; Park, 2016).

In patients with nonalcoholic steatohepatitis (NASH), circulating and tissue levels of FGF21 are increased, correlate with disease severity, and are normalized by therapeutic interventions (Struik, 2019), indicating the presence of FGF21 resistance that is at least in part attributable to downregulation of fibroblast growth factor receptor 1 (FGFR1) and β-Klotho expression in the liver (Oh, 2012).

Administration of exogenous FGF21 is being explored as a method to treat obesity-associated insulin-resistance disorders including NASH, the higher-risk variant of nonalcoholic fatty liver disease (NAFLD). There are currently no approved pharmaceutical treatments for NASH(Friedman, 2018). Several nonclinical and clinical studies have shown that administration of various FGF21 analogues had beneficial effects on serum lipids and insulin-resistance as well as on liver fat, liver enzymes and liver histology (Gaich, 2013; Zhang, 2014; Sanyal, 2019; Kaufman, 2020).

This randomized, double-blind, placebo-controlled Phase 2b study is designed to assess the efficacy, safety, and tolerability of 3 dose regimens of pegozafermin (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 NASH (NAFLD activity score [NAS] ≥4, fibrosis stage F2 or F3 [NASH CRN system]). The study will allow evaluation of the potential histological benefit of pegozafermin in the target population.

Objectives and Endpoints (Primary, Secondary and Safety)

Main Study Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the effect of pegozafermin on liver histology after 24 weeks of treatment	• Proportion of subjects with NASH resolution without worsening of fibrosis[1] at Week 24 compared to baseline. • Proportion of subjects achieving improvement of fibrosis ≥1 stage without worsening of NASH at Week 24 compared to baseline.
Key Secondary	
• To further evaluate the effect of pegozafermin on liver histology after 24 weeks of treatment	• Proportion of subjects with at least a 2-point improvement in NAS and no worsening of fibrosis at Week 24 compared to baseline. • Proportion of subjects with NASH resolution AND fibrosis improvement ≥1 stage at Week 24 compared to baseline. • Proportion of subjects with ≥ 2-point improvement in NAS score AND are MRI-PDFF responders[2] AND ALT responders[3] at Week 24 compared to baseline.
Secondary	
• To evaluate the effects of pegozafermin on liver parameters	• Absolute change and percentage change from baseline at Week 12 and Week 24 in: – Hepatic fat fraction by magnetic resonance imaging - proton density fat fraction (MRI-PDFF) – Alanine aminotransferase (ALT) – N-terminal type III collagen propeptide (Pro-C3)

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

Objectives	Endpoints
• To evaluate the metabolic effects of pegozafermin	• Absolute and percent change from baseline at Weeks 12 and 24 in: – Adiponectin – Serum triglycerides – High density lipoprotein cholesterol (HDL-c) – Non-HDL-c – Low density lipoprotein-cholesterol (LDL-c) – Glycated hemoglobin (HbA1c)
• To characterize pegozafermin pharmacokinetics (PK) profile	• Trough concentration of pegozafermin
Safety	
• To evaluate the safety and tolerability of pegozafermin	• Frequency and severity of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) • Number of subjects who discontinued due to TEAEs and due to related TEAEs • Incidence and shifts of clinically significant physical examination findings, electrocardiogram (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)

Objectives	Endpoints
• To evaluate the immunogenicity of pegozafermin	• Incidence and characteristics of antidrug 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

Extension Study Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the long-term safety and tolerability of pegozafermin	• Frequency and severity of TEAEs and SAEs at Week 48
Secondary	
• To characterize effect of pegozafermin on liver parameters	• Absolute change and percent change from baseline at Week 48 in: – Hepatic fat fraction by MRI-PDFF – ALT – Pro-C3
• To characterize pegozafermin PK profile	• Trough concentration of pegozafermin
Safety	
• To evaluate additional safety and tolerability measures	• 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 – IGF-1

Objectives	Endpoints
	– Bone biomarkers: CTX, P1NP and osteocalcin – TSH • Absolute and % change from baseline in lumbar spine, total hip, and femoral neck BMD as assessed by DXA
• To evaluate the immunogenicity of pegozafermin over time	• Incidence and characteristics of ADA and NAb after dosing (e.g., titer and binding specificity, to the FGF21 and PEG part of pegozafermin) • Impact of the presence of ADAs on serum pegozafermin concentrations and clinical safety • Levels of endogenous FGF21 at immunogenicity follow-up visit(s) compared to baseline in [redacted] subjects

Overall Design

This 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 subjects with biopsy-confirmed NASH (NAS ≥4, fibrosis stage F2 or F3 per NASH Clinical Research Network (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 Section 1.2.

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 Section 1.3 and described in Section 4.1. Throughout the study (Main and Extension), subjects will be evaluated as specified in the SoA. All subjects will be followed-up for at least 4 weeks after last dose of investigational product (IP).

For post-treatment 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 [redacted] AEs and concomitant medications 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.

Main Study

After signing informed consent, subjects will undergo screening assessments to determine eligibility over a period of 12 weeks. In line with the FDA guidance, in instances where COVID-19 pandemic restrictions or logistical scheduling challenges may be of concern, the screening period may be extended beyond 12 weeks with Sponsor approval(FDA, August 2021).

On Day 1 (baseline), eligible subjects 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. The randomization will be stratified by Type 2 diabetes mellitus (T2DM) status (yes vs no) and fibrosis stage (F2 vs F3). For the purposes of stratification, subjects will be considered to be T2DM status positive if any of the following criteria are met: current medical history of T2DM, current use of anti-glycemic medication(s) for T2DM, or screening laboratory values of ≥6.5% for HbA1c or ≥126 mg/dL for fasting plasma glucose. IP will be administered over 24 weeks (includes 24 administrations in QW dose regimen and 12 administrations in Q2W dose regimen). In the Main study, subjects, investigators and site staff, and Sponsor will be blinded to IP (placebo or pegozafermin); however, the dose regimen (QW or Q2W) will be known to all parties involved in the study. Subjects 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 (refer to Section 6.4.3 for details).

Extension Study

Subjects 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. 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) and receive the re-randomized treatment beginning at the Week 26 visit (which may be an in-clinic or a remote home visit). All other subjects 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). 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). Dose regimen (QW or Q2W) will be known to all parties in the Extension study.

Number of Subjects and Intervention Groups

A total of approximately 184 subjects are planned in the Main study as follows:

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

Abbreviations: QW, weekly; Q2W, every 2 weeks; SC, subcutaneously
* 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)

Study Duration

For each subject, the total duration of study participation will be up to approximately 112 weeks:

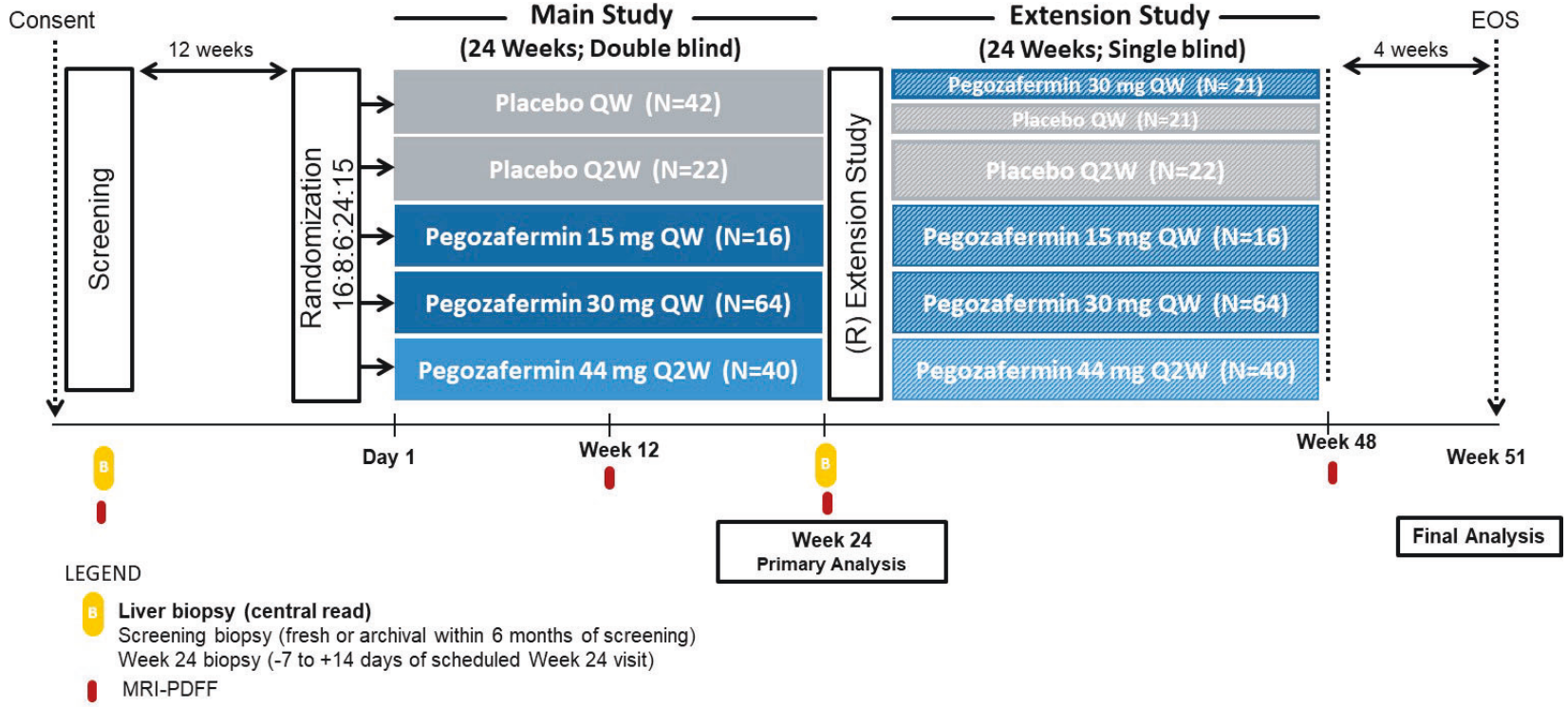
Screening Period:	Up to 12 weeks, which may be extended with Sponsor's approval
Main Study Treatment Period:	24 weeks (includes 24 administrations in QW dose regimen and 12 administrations in Q2W dose regimen)
Extension Study Treatment Period:	24 weeks (includes 24 administrations in QW dose regimen and 12 administrations in Q2W dose regimen)
Extension Study Follow-up Period:	Approximately 4 weeks from last dose of IP.
Immunogenicity Follow-up Period	Approximately [redacted] from last dose of IP

Data Monitoring Committee: Yes

An independent DMC will periodically review overall unblinded safety data per DMC charter.

1.2. Study Schema

Figure 1: BIO89-100-122 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. However, the dose regimen (QW or Q2W) will be known to all parties involved in both the Main and Extension study.
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 [redacted] 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.

1.3. Schedule of Activities (SoA)

Table 1: Schedule of Activities

Subjects will attend clinic visits for Screening, Baseline (Day 1), Week 4, Week 12, Week 24, Week 38 and Week 48/ET.

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:
		Main Study						Extension Study						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
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 circumference	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 VCTE 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:
		Main Study						Extension Study						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
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 factors	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		
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

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	
														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 outcomes		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).
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

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	
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. 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. **o.** Week 26 may be an in-clinic or a remote/home visit by healthcare professional who will administer the IP.
			Q2W from Weeks 2 to 22				Q2W from Weeks 24 to 46							
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.
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

Assessments	Screening Period[1]	Treatment Period										FU Period		Notes:
		Main Study						Extension Study						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
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	
														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 requirements for values >15 nmol/L on treatment (Section 10.2).
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

Assessments	Screening Period[1]	Treatment Period										FU Period		Notes:
		Main Study						Extension Study						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
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	
														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 analysis		B			X		X				X	X		Includes CTX, P1NP, and osteocalcin; refer to Appendix 2 (Section 10.2).
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 Table 2). Endogenous FGF21 samples (baseline and follow-up visit) will be analyzed only in subjects with neutralizing ADAs.
Immunogenicity		B	X	X	X		X			X	X[t]	X		**t.** See Table 2 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 medications	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

Table 2: 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 [redacted]. 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

2. INTRODUCTION

2.1. Study Rationale

BIO89-100 (hereafter referred to as pegozafermin), developed by 89bio, is a glycoPEGylated analogue of fibroblast growth factor 21 (FGF21), an endogenous metabolic hormone secreted by the liver, adipose tissue, skeletal muscle, and the pancreas, that is regulated by nutritional status and affects energy expenditure and glucose and lipid metabolism (Arner, 2008; Lee, 2014; Park, 2016).

In patients with nonalcoholic steatohepatitis (NASH), circulating and tissue levels of FGF21 are increased, correlate with disease severity, and are normalized by therapeutic interventions (Struik, 2019), indicating the presence of FGF21 resistance that is at least in part attributable to downregulation of fibroblast growth factor receptor (FGFR1) and β-Klotho expression in the liver (Oh, 2012).

Administration of exogenous FGF21 is being explored as a method to treat obesity-associated insulin-resistance disorders including NASH, the higher-risk variant of nonalcoholic fatty liver disease (NAFLD). There are currently no approved pharmaceutical treatments for NASH (Friedman, 2018). Several nonclinical and clinical studies have shown that administration of various FGF21 analogues had beneficial effects on serum lipids and insulin-resistance as well as on liver fat, liver enzymes and liver histology (Gaich, 2013; Zhang, 2014; Sanyal, 2019; Kaufman, 2020).

This randomized, double-blind, placebo-controlled Phase 2b study is designed to assess the efficacy, safety and tolerability of 3 dose regimens of pegozafermin (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 NASH (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 in the target population.

2.2. Background

NASH is a chronic liver disease, characterized histologically by ≥5% hepatic steatosis, inflammation and hepatocellular injury (ballooning) (Chalasani, 2012). It is part of the spectrum of NAFLD, which is the most common chronic liver disease in North America and Europe. The NAFLD spectrum ranges from bland steatosis with or without inflammation (nonalcoholic fatty liver) to steatosis plus inflammation and hepatocellular injury, i.e., NASH, fibrosis and cirrhosis (Estes, 2018; Chen, 2020).

The pathophysiologic mechanisms that lead to NAFLD are thought to be similar to those in the development of type 2 diabetes mellitus (T2DM), as insulin resistance is a hallmark in both disease states. Insulin resistance in adipocytes leads to dysfunction in the normal regulators of lipolysis, contributing to elevated circulating free fatty acids, accumulation of triglycerides in the liver (Saponaro, 2015) and lipotoxicity, manifested as inflammation and cellular injury (Byrne, 2015). It is unclear why some patients with NAFLD do not progress to NASH; some genetic modifiers of disease progression and severity have been identified (including patatin-like phospholipase domain-containing 3 (PNPLA3) (Dongiovanni, 2013). T2DM is associated with a greater than 2-fold increase in the risk of developing severe liver disease (Jarvis, 2020).

NAFLD is usually asymptomatic unless progression to cirrhosis has occurred. It is often diagnosed by demonstration of hepatic steatosis on liver imaging (e.g., ultrasound or magnetic resonance imaging [MRI]) in subjects, commonly with features of the metabolic syndrome (MetS), in whom no alternative etiology for liver fat accumulation can be identified (e.g., alcoholic liver disease, medications). A liver biopsy is the gold standard for assessing the presence of NASH and the presence of advanced fibrosis (the most important prognostic indicator in subjects with NAFLD) (Dulai, 2017; Balakrishnan, 2020); however, a number of non-invasive tools, e.g., clinical risk scores such as the NAFLD fibrosis score (NFS) and Fibrosis-4 (FIB-4) index, the enhanced liver fibrosis (ELF) score, and vibration-controlled transient elastography (VCTE), have been validated and are increasingly used in clinical settings for differentiation between subjects with a high vs. low risk of advanced fibrosis (EASL-EASD-EASO, 2016; Chalasani, 2018; Leoni, 2018).

The global prevalence of NAFLD in the general adult population has been estimated to be ~25% in a large meta-analysis of studies published through 2015 (Younossi 2016). The highest prevalence rates were reported in the Middle East and South America, and the lowest were in Africa. In North America and Europe, the prevalence of NAFLD is estimated at 21-25% (Younossi, 2016; Arshad, 2020; Mitra, 2020). The global prevalence of NASH has been estimated to range from 3% to 5% (Younossi, 2016; Younossi, 2018). The prevalence of NAFLD is on the rise (Younossi, 2016); in the US, the number of NAFLD cases is projected to expand from 83.1 million in 2015 (~25% of the population) to 100.9 million in 2030. An increased proportion of these cases will be NASH, rising from 20% to 27% of adults with NAFLD during this interval (Estes, 2018).

There is a high unmet medical need in NASH (Friedman, 2018). The disease progresses to fibrosis and cirrhosis in approximately 20% of affected patients (Vernon, 2011; Khan, 2015), and 45% of affected patients with cirrhosis will progress to decompensated cirrhosis within 10 years of diagnosis (Rinella, 2015). Eight percent of patients with advanced fibrosis will develop hepatocellular carcinoma within 5 years (Hashimoto, 2009), and there is increasing evidence that non-cirrhotic subjects with NAFLD may also be at an increased risk of hepatocellular carcinoma (Mittal, 2016). Moreover, NASH-related cirrhosis has become one of the leading causes of liver transplantation (Charlton, 2011; Golabi, 2018; Noureddin, 2018; Shingina, 2019).

As of this time, there are no approved therapies for treatment of patients with NASH (Attia, 2020). A variety of therapeutic agents are being developed for NASH, targeting metabolic pathways, inflammatory pathways or fibrosis (Attia, 2020; Dewidar, 2020; Dufour, 2020). Positive interim results (reduction of fibrosis) were reported from the ongoing REGENERATE Phase 3 study with obeticholic acid in patients with liver fibrosis due to NASH (Younossi, 2019a). In a Phase 2a proof-of-concept study, positive data were reported in NASH patients treated with pharmacological doses of FGF21 for 16 weeks with BMS-986036 (Sanyal, 2019; Verzijl, 2020). Furthermore, a beneficial effect of an Fc-FGF21 analog efruxifermin (AKR-001) was reported on key NASH parameters, e.g., reduction of liver fat and evaluation of histologic changes at 5 months in an ongoing study (BALANCED) in NASH patients (Akero Therapeutics Inc, 2020). In a Phase 1b/2a study (BIO89-100-002, Part 1), repeat administration of multiple

ascending doses of pegozafermin in subjects with NASH or phenotypic NASH (PNASH[4]) led to robust, significant, and clinically meaningful benefits across key liver parameters observed across all dose groups with concurrent metabolic benefits (Loomba, 2020b). Open-label, initial assessment of the effect of pegozafermin on histological endpoints in approximately 20 subjects with biopsy-proven NASH (F1 with high risk, F2 and F3) is ongoing (BIO89-100-002, Part 2).

2.3. Benefit / Risk Assessment

Information about the expected benefits and potential risks (adverse events) of pegozafermin can be found in the Investigator's Brochure (IB).

2.3.1. Risk Assessment

The safety of pegozafermin has been assessed in Phase 1a single ascending dose (dose range 0.45 to 78 mg) study in healthy volunteers (TV47948-SAD-10122) and Phase 1b/2a multiple ascending dose (dose range 3 to 27 mg QW, 18 mg Q2W and 36 mg Q2W) study in subjects with NASH, or with NAFLD who are at high risk of NASH (BIO89-100-102 Part 1). pegozafermin was generally well tolerated in both studies.

In a Phase 1b/2a study (BIO89-100-102 Part 1), repeat administration of multiple ascending doses of pegozafermin in both NASH and PNASH subjects was generally well tolerated and associated with a favorable safety profile per treatment-emergent adverse events (TEAEs), laboratory (chemistry, hematology, and urinalysis), vital signs and electrocardiogram (ECG) findings. There were no deaths or treatment-related serious adverse events (SAEs); one subject discontinued treatment due to a treatment-related TEAE, a Grade 2 drug eruption. The most common TEAEs, occurring in 5% or more of subjects, were increased appetite, diarrhea, headache and nausea. The incidence of gastrointestinal (GI) TEAEs was comparable between pooled pegozafermin and placebo with no differences between dose groups in incidence of individual GI TEAEs and total TEAEs for the GI system organ class (SOC). There was no clear correlation between frequency of TEAEs and dose. No bone or female reproductive tract-related adverse events (AEs) were reported (spontaneous reporting). There were no consistent patterns of any abnormalities in safety parameters that appeared to be attributable to pegozafermin. Overall, no safety concern was identified associated with pegozafermin.

Information on important identified and potential risks associated with pegozafermin is provided in the IB.

2.3.2. Benefit Assessment

There are currently no approved products for NASH in the US.

Patients with biopsy-confirmed NASH may derive benefit from treatment with pegozafermin.

In Part 1 of Study BIO89-100-002, repeat administration of ascending doses of pegozafermin (3 mg, 9 mg, 18 mg and 27 mg QW and 18 mg and 36 mg Q2W) for up to 12 weeks in subjects with NASH or PNASH led to robust, significant, and clinically meaningful benefits in key liver parameters observed across all dose groups, with concurrent metabolic benefits. All dose groups

[4] Phenotypic NASH, defined as at least one of the following: 1) obesity with T2DM. 2) Obesity with evidence of liver injury (either increased alanine aminotransferase [ALT] and/or vibration-controlled transient elastography [VCTE] score ≥7 KPa)

demonstrated significant reductions in liver fat (up to 60% reduction compared to baseline; up to 70% reduction compared to placebo), and liver volume (up to 15% reduction) as measured by magnetic resonance imaging-estimated proton density fat fraction (MRI-PDFF). A significant proportion of subjects responded to therapy, with up to 88% and 71% of subjects achieving a ≥30% or a ≥50% reduction in liver fat versus baseline, respectively. Treatment with pegozafermin also resulted in significant improvements in liver transaminases [up to 44% reduction in alanine aminotransferase (ALT), 38% reduction in aspartate aminotransferase (AST)], and in N-terminal type III collagen propeptide (Pro-C3), a marker of fibrosis (up to 28% reduction) following 12 weeks of treatment. Of note, reduction of liver fat ≥30% and ALT decrease ≥17 U/L or ≥30% have been previously reported to be associated with positive histological outcomes (Harrison, 2020b; Loomba, 2020c). Metabolic benefits of pegozafermin included a favorable effect on lipids, with significant reductions in triglycerides (up to 28% reduction), non-high density lipoprotein (non-HDL) and low density lipoprotein-cholesterol (LDL-c) (up to 16% reduction), and significant increases in the insulin sensitizing hormone adiponectin (up to 61%). All these effects were observed in both dose regimens evaluated (QW and Q2W) and were most prominent at the 27 mg QW dose and the 36 mg Q2W dose. There were no differences between population groups evaluated (biopsy-confirmed NASH or PNASH).

Based on these findings, it is estimated that 24 weeks of treatment with pegozafermin may have a positive impact on liver histology, liver-related (e.g., reduction of hepatic fat) and metabolic parameters in subjects with biopsy-confirmed NASH.

2.3.3. Overall Benefit Risk Assessment

Considering the benefit, potential risks, and risk mitigation measures that have been implemented, the Sponsor considers the benefit-risk profile for administering pegozafermin to subjects with biopsy-confirmed NASH in study BIO89-100-122 to be favorable. The overall benefit-risk associated with administration of pegozafermin will be continually re-assessed with the emergence of additional data.

3. OBJECTIVES AND ENDPOINTS

3.1. Main Study Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the effect of pegozafermin on liver histology after 24 weeks of treatment	• Proportion of subjects with NASH resolution without worsening of fibrosis[5] at Week 24 compared to baseline. • Proportion of subjects achieving improvement of fibrosis ≥1 stage without worsening of NASH at Week 24 compared to baseline.
Key Secondary	
• To further evaluate the effect of pegozafermin on liver histology after 24 weeks of treatment	• Proportion of subjects with at least a 2-point improvement in NAS and no worsening of fibrosis at Week 24 compared to baseline. • Proportion of subjects with NASH resolution AND fibrosis improvement ≥1 stage at Week 24 compared to baseline. • Proportion of subjects with ≥ 2-point improvement in NAS score AND are MRI-PDFF responders[6] AND ALT responders[7] at Week 24 compared to baseline.
Secondary	
• To evaluate the effects of pegozafermin on liver parameters	• Absolute change and percentage change from baseline at Week 12 and Week 24 in: – Hepatic fat fraction by MRI-PDFF – ALT – Pro-C3

[5] Resolution of NASH includes the total absence of ballooning (score=0) and absent or mild inflammation (score 0 to 1).
Worsening of fibrosis is defined as progression of fibrosis ≥ 1 stage.
Worsening of NASH is defined as increase in NAS for ballooning, inflammation, or steatosis
[6] ≥30% reduction from baseline in liver fat by MRI-PDFF
[7] ≥17 U/L or ≥30% reduction from baseline in ALT

Objectives	Endpoints
• To evaluate the metabolic effects of pegozafermin	• Absolute and percent change from baseline at Weeks 12 and 24 in: – Adiponectin – Serum triglycerides – High density lipoprotein cholesterol (HDL-c) – Non-HDL-c – LDL-c – Glycated hemoglobin (HbA1c)
• To characterize pegozafermin pharmacokinetics (PK) profile	• Trough concentration of pegozafermin
Safety	
• To evaluate the safety and tolerability of pegozafermin	• 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)
• To evaluate the immunogenicity of pegozafermin	• Incidence and characteristics of anti-drug antibodies (ADA) and neutralizing antibody (NAb) after dosing (e.g., titer

Objectives	Endpoints
	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
Exploratory	
• To evaluate effect of pegozafermin on additional clinical parameters	Change from baseline in the following clinical parameters: **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 improvement of fibrosis ≥1 stage without worsening of NASH • Proportion of subjects with NASH resolution without worsening of fibrosis and ≥ 2-point improvement in NAS score at Week 24 compared to baseline. • Proportion of subjects achieving improvement of fibrosis ≥1 stage without worsening of NASH and ≥ 2-point improvement in NAS score at Week 24 compared to baseline. • 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

Objectives	Endpoints
	• Fibrosis improvement ≥1 stage without worsening of NASH AND are MRI-PDFF responders • 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 Mean change from baseline in: • NAS score • Fibrosis score (categorical) In MRI-PDFF responders[8]: Proportion of subjects 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 – Mean change from baseline in NAS score • Artificial intelligence (AI)-based histological analysis (PathAI)

[8] ≥30% reduction from baseline in liver fat by MRI-PDFF

	Imaging assessments • Hepatic fat content – 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 • Change in hepatic fibro-inflammation as measured by Iron-corrected T1 (cT1) imaging • Change in FibroScan VCTE score (liver stiffness measurement) and CAP (steatosis) • Change in liver and spleen volume • Change in pancreatic fat percentage **Anthropometric assessments** • Body weight • Waist circumference • Waist/hip ratio **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) • ELF score

Objectives	Endpoints
	• FIB-4 index
• To evaluate the effects of pegozafermin on patient reported outcome (PRO)	• Change from baseline scores at Weeks 12 and 24 in: – Chronic Liver Disease Questionnaire-NAFLD-NASH (CLDQ NAFLD-NASH) – Work Productivity and Activity Impairment 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 VAS
• 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)	• To identify emerging biomarkers from RNA/DNA, plasma and serum samples collected • Effect of naturally occurring genetic variation on the efficacy (e.g., clinical remission, clinical response), safety, and/or PK profile with pegozafermin

3.2. Extension Study Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the long-term safety and tolerability of pegozafermin	• Frequency and severity of TEAEs and SAEs at Week 48
Secondary	
• To characterize effect of pegozafermin on liver parameters	• Absolute change and percent change from baseline at Week 48 in: – Hepatic fat fraction by MRI-PDFF – ALT – Pro-C3

Objectives	Endpoints
• To characterize pegozafermin PK profile	• Trough concentration of pegozafermin
Safety	
• To evaluate additional safety and tolerability measures	• 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 – IGF-1 – Bone biomarkers: CTX, P1NP and osteocalcin – TSH • Absolute and % change from baseline in lumbar spine, total hip, and femoral neck bone mineral density (BMD) as assessed by DXA
• To evaluate the immunogenicity of pegozafermin over time	• Incidence and characteristics of ADA and NAb after dosing (e.g., titer and binding specificity, to the FGF21 and 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 [redacted] subjects
Exploratory	
• To evaluate pegozafermin on additional metabolic parameters	• Mean and percent change from baseline at Weeks 38 and 48 in: – Adiponectin – Serum triglycerides

Objectives	Endpoints
	– HDL-c – Non-HDL-c – LDL-c – HbA1c
• To evaluate pegozafermin on additional clinical parameters	Change from baseline in the following clinical parameters: **Imaging assessment** • Change in hepatic fibro-inflammation as measured by cT1 imaging • Change in FibroScan VCTE score (liver stiffness measurement) and CAP (steatosis) **Anthropometric assessments** • Body weight • Waist circumference • Waist/hip ratio **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
• To evaluate the effects of pegozafermin on PRO	• Change from baseline scores at Week 48 in:

Objectives	Endpoints
	– CLDQ NAFLD-NASH – WPAI-NASH – EQ-5D-5L – Liver-related pain and discomfort questionnaire – The appetite sensations VAS

4. STUDY DESIGN

4.1. Overall Study Design

This 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 subjects 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 Section 1.2.

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 Section 1.3.

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) 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.

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 [redacted]. 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.

A Data Monitoring Committee (DMC) will periodically convene to review unblinded overall safety results per DMC charter (Section 9.8).

Main Study

After signing informed consent, subjects will undergo screening assessments to determine eligibility over a period of 12 weeks. Eligibility period may be extended with Sponsor's approval. Individual screening assessments may be done remotely as part of tiered screening. Subjects will either undergo a liver biopsy during Screening or have a recent liver biopsy (within 6 months prior to consent, available to be evaluated for eligibility by the central reader) that meets study inclusion criteria; ideally, the liver biopsy should be done after all non-invasive eligibility criteria are met.

On Day 1 (baseline), eligible subjects will be randomized to one of the following treatment groups:

- Placebo, N=64; QW (N=42) or Q2W (N=22)
- Pegozafermin 15 mg, QW subcutaneous (SC), N=16
- Pegozafermin 30 mg, QW, SC, N=64
- Pegozafermin 44 mg, Q2W, SC, N=40

with the randomization 16:8:6:24:15 to Placebo QW, Placebo Q2W, pegozafermin 15 mg QW, 30 mg QW and 44 mg Q2W, respectively. The randomization will be stratified by T2DM status (yes vs. no) and fibrosis stage (F2 vs. F3). For the purposes of stratification, subjects will be considered to be T2DM status positive if any of the following criteria are met: current medical history of T2DM, current use of anti-glycemic medication(s) for T2DM, or screening laboratory values HbA1c ≥6.5% or fasting plasma glucose ≥126 mg/dL. Subjects will receive IP over 24 weeks (includes 24 administrations in QW dose regimen and 12 administrations in Q2W dose regimen). Subjects, investigators and site staff and Sponsor will be blinded to IP (placebo or pegozafermin); however, the dose regimen (QW or Q2W) will be known to all parties involved in the study.

Subjects 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 (refer to Section 6.4.3 for details).

Extension Study

Subjects 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.

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) and receive the re-randomized treatment at the Week 26 visit (which may be an in-clinic or a remote home visit). All other subjects 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.

4.2. Scientific Rationale for Study Design

Data from BIO89-100-002 Part 1 demonstrated favorable safety and tolerability with repeated dosing of pegozafermin for 12 weeks. In addition, robust, clinically meaningful reductions in liver fat, assessed by MRI-PDFF, and ALT, at thresholds that have been associated with histological benefit in subjects with NASH, have been observed.

This study will further evaluate subjects with biopsy-confirmed NASH and fibrosis (NAS ≥4, fibrosis stage F2 or F3 per NASH CRN system), similar to the population that is typically enrolled in Phase 2b and pivotal Phase 3 NASH studies, who will be treated with pegozafermin

for approximately 48 weeks (with ~24 weeks in the Main study and ~24 weeks in the Extension study). The study will be placebo-controlled throughout its duration, double-blinded in the Main study and single-blinded in the Extension study. Specifically-identified Sponsor personnel will be unblinded after the Main study is completed to evaluate the data and prepare for interactions with the FDA. However, subjects, Investigators, site staff, and Sponsor personnel responsible for day-to-day study operations including the Sponsor and CRO Medical Monitors, will remain blinded to treatment assignment throughout the entire study in order to maintain the integrity of the primary endpoint (safety) of the Extension study.

The primary endpoints of the Main study will further assess the effect of different doses/regimens of pegozafermin on improvement in liver histological endpoints compared to baseline at Week 24, including, 1) NASH resolution without worsening of fibrosis and ≥ 2-point improvement in NAS score, 2) improvement of fibrosis ≥1 stage without worsening of NASH and ≥ 2-point improvement in NAS score. These modified histological primary endpoints are consistent with the FDA guidance on endpoints likely to predict clinical benefit and incorporate NAS improvement as a measure that reflects decrease in disease severity, thus is clinically meaningful (Neuschwander-Tetri, 2015; Harrison, 2019; FDA Draft Guidance for Industry, December 2018). Other Main study secondary efficacy endpoints include changes in liver fat, other histological endpoints, and additional liver and metabolic biomarkers. The study will contribute to further characterization of the safety profile for pegozafermin. Patient reported outcomes and other exploratory endpoints will also be assessed. Efficacy data from this study, in particular findings related to the effect of pegozafermin at the studied doses and dose regimens on the assessed histological endpoints, will support dose selection and endpoint determination in the anticipated Phase 3 program.

The Extension study will allow long-term safety (including long-term immunogenicity) and evaluation of noninvasive biomarker assessments in a single-blinded manner. The re-randomization of subjects assigned to placebo QW treatment arm in Main study to receive either active (pegozafermin 30 mg QW) or placebo QW will allow replication of the pegozafermin effects on noninvasive biomarker results observed in the Main study. The two-week delay in treatment change for this group will ensure that all biopsies are performed prior to any of these subjects being switched from placebo to IP.

4.3. Justification for Dose

The dose levels and regimens for this study, pegozafermin QW (15 mg or 30 mg) or Q2W (44 mg), were selected based on nonclinical safety and pharmacology studies in mice and monkeys, safety and pharmacodynamic data from the single ascending dose (SAD) study TV47948-SAD-10122, and data from the multiple ascending dose (MAD) study BIO89-100-002 Part 1.

In Part 1 of Study BIO89-100-002, exposures to pegozafermin (ascending doses 3 mg, 9 mg, 18 mg, and 27 mg QW and 18 mg and 36 mg Q2W) in subjects with NASH and PNASH for up to 12 weeks were generally dose-proportional following QW or Q2W dosing. Exposures were related to the total doses regardless of dosing regimens. Terminal phases of concentration-time profiles were parallel, suggesting a dose-proportional PK. AUC_{last} values stratified by regimens are also generally dose-proportional. Group t_{max} occurred between 48 and 72 hours after dosing. Accumulation ratios ranged from 1.0 to 1.4.

Exposure-response relationship based on Week [redacted] was established using data from Study BIO89-100-002 (Part 1). The data show that higher exposures were associated with better response and can be described by an E_{max} (maximum effect) model. The high dose proposed for this study, 30 mg QW, likely approaches full effect. The proposed low dose, 15 mg QW, is expected to elicit close to or slightly better than EC50 effect. The 44 mg Q2W dose (roughly equivalent to 22 mg, QW) is proposed as a middle dose.

The predicted $AUC_{(0\text{-dosing interval})}$ for the highest dose in Study BIO89-100-122 was compared to exposure at the no observed adverse effect level (NOAEL) [redacted] in the 26-week chronic toxicity study in mice to determine safety margins. Clinical PK parameters obtained from BIO89-100-002 were used for prediction. When using the predicted $AUC_{(0\text{-dosing interval})}$ at steady state for 30 mg QW and 44 mg Q2W, the highest doses in BIO89-100-122, safety margins were calculated to be [redacted] and [redacted], respectively. Given that the adverse effects that determined the NOAEL at [redacted] in this study are considered to be secondary to very significant body weight reduction in mice that was not observed with pegozafermin in human subjects with NASH or PNASH who were treated at a similar dose for up to [redacted] weeks, and the uncertain clinical relevance of the adverse findings in lean mice to mostly overweight or obese human subjects, this safety margin is considered acceptable.

Based on these data, the selected doses and dose regimens are anticipated to be safe, of sufficient duration to reach steady state, maintain PD responses over the dosing intervals, and cover the dose range where key PD responses are predicted to be associated with important clinical outcomes to be assessed in subsequent studies. The results of this study are expected to provide valuable information to guide dose selection for future, pivotal pegozafermin studies with histological endpoints in NASH.

4.4. Study Duration

For each subject, the total duration of study participation will be up to approximately 112 weeks:

Screening Period:	Up to 12 weeks, which may be extended with Sponsor's approval
Main Study Treatment Period:	24 weeks (includes 24 administrations in QW dose regimen and 12 administrations in Q2W dose regimen)
Extension Study Treatment Period	24 weeks (includes 24 administrations in QW dose regimen and 12 administrations in Q2W dose regimen)
Extension Study Follow-up Period	Approximately 4 weeks from last dose of IP
Immunogenicity Follow-up Period	Approximately [redacted] from last dose of IP

4.5. End of Study and End of Immunogenicity Follow-up Period Definition

A subject is considered to have completed the study if he or she has completed the EOS follow-up visit (Week 51/EOS) or has undergone early termination. The end of the study is defined as the date of the last visit (EOS visit) of the last subject in the study. The end of the

immunogenicity follow-up period is defined as the last follow-up visit for the immunogenicity period.

5. ELIGIBILITY CRITERIA

The screening process is designed to minimize unnecessary biopsies by identifying subjects at high risk for NASH with Stage 2 or 3 fibrosis (per NASH CRN). All efforts should be made to ensure that subjects complete the noninvasive assessments prior to the screening biopsy. See Appendix 7 (Section 10.7) for Sponsor guidance on prescreening potential subjects that may be at high risk for NASH with Stage 2 or 3 fibrosis (NASH CRN).

5.1. Inclusion Criteria

Subjects are eligible to be included in the study only if all of the following criteria apply:

Age and Gender

1. Subjects must be 21 to 75 years of age inclusive, at the time of signing the informed consent form (ICF).
2. Male or female.

Type of Subject and Disease Characteristics

3. Biopsy-confirmed NASH with fibrosis stage F2, or F3 per NASH CRN system and NAS ≥4, with a score of at least 1 in each of steatosis, ballooning degeneration, and lobular inflammation, either through a historical biopsy or a biopsy at screening. A historical biopsy should be obtained within 6 months prior to first day of Screening (i.e., day ICF is signed) that is deemed suitable for interpretation by a central reader if the subject had no significant change in metabolic status (control of diabetes, hyperlipidemia or >5% weight loss or gain).

Pregnancy and Contraception

Contraceptive use by men or women should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies. Refer to Appendix 4 (Section 10.4) for definitions of women of childbearing potential (WOCBP) and contraception guidance.

4. All subjects (male or female) who are of childbearing potential must agree to use highly effective, double contraception (both male and female partners) during the study. Use of a condom with spermicide in a male subject who underwent vasectomy is also acceptable as double contraception. Use of highly effective, double contraception must continue for 30 days after the last dose of IP. Female subjects should not donate oocytes during this time. Male subjects must not donate sperm during this time. Rhythm methods are not considered as highly effective methods of birth control. Subject abstinence for the duration of the study and 30 days after the last dose of IP is acceptable if it is the subject's regular practice.
5. Females of childbearing potential must have a negative serum pregnancy test at Screening and a negative urine pregnancy test on Day 1. Females of childbearing

potential must agree to undergo a pregnancy test prior to dosing at the timepoints specified in the SoA.

6. Sexually active male subjects whose female partner is pregnant must agree to use a condom.

Informed Consent and Study Requirements

7. Capable of giving signed informed consent, which includes compliance with the requirements and restrictions listed in the ICF and in this protocol.
8. Subjects must not participate in any other interventional studies throughout the duration of this study. COVID-19 protocols may be excepted with Medical Monitor (or designee) approval.

5.2. Exclusion Criteria

Subjects are excluded from the study if any of the following criteria apply:

Medical Conditions

Liver Disease

1. History of a liver disorder other than NASH or clinical suspicion of a liver disorder other than NASH, including but not limited to hepatitis B and hepatitis C, autoimmune hepatitis, hemochromatosis, alcoholic liver disease, primary sclerosing cholangitis, primary biliary cirrhosis/cholangitis, alpha-1 antitrypsin deficiency, untreated celiac disease, or Wilson's disease.

   Serology testing will be performed at screening. Subjects positive for hepatitis B surface antigen (HBsAg) will be excluded. Subjects positive for hepatitis C virus antibody (antiHCV) will undergo reflex polymerase chain reaction (PCR) for HCV RNA, and will only be eligible if the following conditions are met:

   a. Subjects with spontaneous clearance of HCV infection (positive serology for HCV infection with documented negative PCR for HCV RNA and no history of acute HCV infection within 3 years prior to Screening).
   b. Subjects who were previously diagnosed with chronic HCV infection who achieved documented sustained viral response (SVR) following treatment at least 2 years prior to Screening.

2. Planned or history of liver transplantation.
3. History or evidence of **cirrhosis** (NASH CRN Fibrosis Stage 4 on biopsy) or clinical indicators of hepatic decompensation including ascites, hepatic encephalopathy, splenomegaly, or variceal bleeding.

Other Medical Conditions

4. Presence of any **chronic medical condition** that, in the opinion of the Investigator, might pose additional risk to the subject, make the subject unable to comply with the protocol requirements, or confound the results of the study. Individual cases in which the Investigator deems the subject appropriate for inclusion despite a clinically significant

chronic medical condition should be discussed with and approved by the Medical Monitor (or designee).

5. Hospitalization due to **COVID-19** within 3 months prior to Screening. A positive COVID-19 test or COVID-19 diagnosis after signing consent is not exclusionary.
6. Human immunodeficiency virus (**HIV)-1 or HIV-2** infection.
7. Unstable or clinically significant **cardiovascular or cerebrovascular disease**:
    a. Unstable angina, myocardial infarction, coronary artery bypass graft (CABG) surgery, percutaneous coronary intervention (PCI), transient ischemic attack (TIA) or cerebrovascular accident (CVA) within 6 months prior to Screening.
    b. Symptomatic valvular or other structural heart disease.
    c. Symptomatic congestive heart failure.
    d. Symptomatic, uncontrolled or high-risk arrhythmia or genetic predisposition to high-risk arrhythmia in the subject or a first degree relative.
    e. Implanted defibrillator or pacemaker.
    f. High risk abdominal aortic aneurysm, uncontrolled peripheral vascular disease, or symptomatic carotid stenosis.
8. Uncontrolled or newly diagnosed (<2 months since diagnosis at time of Screening) **hypertension**. Subjects with well controlled hypertension who are clinically stable may enroll if they have been on a stable dose of antihypertensive medications for at least 2 months before Screening.
9. Uncontrolled or newly diagnosed **thyroid disease**. Subjects with treated thyroid disease may be enrolled if they are considered stable on treatment for at least 3 months by the Investigator. Modest dose adjustments per standard of care are allowed.
10. Uncontrolled or newly diagnosed (≤3 months since diagnosis) **T2DM**[9]:
    a. Subjects must have HbA1c level ≤9.5% at screening.
    b. Subjects must have been on a stable antidiabetic regimen for at least 3 months (for insulin and dipeptidyl peptidase IV [DPP-IV] antagonists) or 6 months (for glucagon-like peptide 1 [GLP-1] agonists and sodium glucose co-transporter 2 [SGLT2] inhibitors) prior to biopsy (historical or screening) and remains stable up to randomization. Stable regimen is defined as no addition or discontinuation of antidiabetic medications, but dose adjustments or switching to another medication in the same class at the same relative dose per standard of care are allowed. Thiazolidinediones are not allowed. Subjects on any other antidiabetic regimen not specified above should be on stable treatment for at least 3 months prior to their qualifying biopsy. Consult with the Medical Monitor if further clarification is needed.
11. Type 1 Diabetes Mellitus.
12. **Weight change** of more than 5% within 3 months prior to on-study screening liver biopsy or more than 10% within 6 months prior to on-study screening liver biopsy or planning to start a new weight loss program, training for a marathon, or taking weight loss medication (See Section 5.3 Lifestyle Considerations). However, in subjects with a

[9] Subjects with newly diagnosed T2DM may be rescreened if considered stable after 3 months.

historical biopsy, weight change of no more than 5% is allowed between the historical biopsy and first day of Screening.

13. History of **bariatric surgery** within the 5 years prior to Screening or plan to have bariatric surgery during conduct of study. Reversible procedures, such as lap banding, are allowed if they have been removed at least 12 months prior to Screening. Note: Removal of intra-gastric balloon or unsuccessful surgery more than 2 years prior to screening is acceptable.
14. History of **bone** trauma, bone fracture, or bone surgery within 2 months of screening or other bone disorders that may have a clinically meaningful impact on bone formation or bone remodeling (such as osteoporosis, osteomalacia) or known, untreated severe vitamin D deficiency (serum 25-hydroxy-vitamin D ≤5 ng/mL; severe vitamin D deficiency that is being treated is not exclusionary). Joint or connective tissue disorders (such as arthritis) are not exclusionary.
15. History of **malignancy** diagnosed or treated within 2 years of screening (recent localized treatment of squamous or noninvasive basal cell skin cancers is permitted; any carcinoma *in situ* is allowed if appropriately treated within 2 years prior to the Screening biopsy); subjects under evaluation for malignancy are not eligible. Any history of hepatocellular carcinoma is exclusionary.
16. Current or history of significant **alcohol consumption** for a period of more than 3 consecutive months within 1 year prior to Screening. Defined as more than 14 units/week for females (>1 drink per day) and more than 21 units/week for males (>2 drinks per day) on average, where one unit of alcohol is equivalent to a 12-oz beer, 4-ounce glass of wine, or 1-ounce shot of hard liquor.
17. History of **substance use disorder**, or any other substance dependence (with the exception of caffeine or nicotine) as defined by the latest edition of the Diagnostic and Statistical Manual of Mental Disorders in the past 2 years prior to Screening. A positive urine drug screen is not exclusionary. Subjects who have a positive test during Screening, including subjects without a history of substance use disorder or subjects who have been prescribed medication (e.g., opiates, benzodiazepines) will be considered for enrollment at Investigator's discretion. Cannabis and cannabidiol (CBD) products are not exclusionary.

Diagnostic Assessments

18. Clinically significant laboratory abnormality at Screening. Repeat tests may be allowed for each laboratory parameter at the discretion of the Investigator. The presence of one or more of the following laboratory abnormalities should lead to exclusion of the subject from participating in the study:
    a. ALT or AST ≥250 U/L
    b. Alkaline Phosphatase >2-fold higher than ULN.
    c. Elevation of total bilirubin (TB) > 1.30 mg/dL. Subjects with isolated indirect hyperbilirubinemia (normal direct bilirubin) secondary to medically documented Gilbert's syndrome may be enrolled
    d. Triglycerides >1000 mg/dL

e. International normalized ratio (INR) > 1.30 unless due to anti-coagulant therapy. Subjects on anti-coagulant therapy may require their treatment withheld according to local guidelines prior to liver biopsy.
f. Glomerular filtration rate (eGFR) ≤ [redacted] mL/min/1.73 m^2 as estimated by chronic kidney disease-epidemiology (CKD-EPI) Creatinine equation (Levey, 2009)
g. Platelet count <100,000/µL
h. Greater than 40% increase in ALT or AST between 2 screening assessments, to be done at least 2 weeks apart between the 1st and 2nd assessment, as per the table below. A 3rd assessment, if required, will be collected via unscheduled visit, and performed at least 1 week apart from the 2nd assessment:

ALT and AST Screening Assessments			Eligibility Status
Assessment 1	**Assessment 2**	**Assessment 3 (if applicable)**	
Normal	Normal	Not applicable	Eligible
Normal	Abnormal and ≤40% increase from Assessment 1	Not applicable	Eligible
Normal	Abnormal and >40% increase from Assessment 1	Normal or ≤40% increase from Assessment 1	Eligible
		Abnormal and >40% increase from Assessment 1	**Excluded**
Abnormal	≤40% increase from Assessment 1	Not applicable	Eligible
Abnormal	>40% increase from Assessment 1	≤40% increase from Assessment 1	Eligible
		>40% increase from Assessment 1	**Excluded**

Normal is defined as ≤ ULN; abnormal is defined as > ULN (upper limit of normal).
Note: Clinical judgment should be used for subjects with isolated AST increases in whom there is suggestion of another cause of AST increase (e.g., muscle injury as evident by concurrent creatine phosphokinase elevation).

19. ECG abnormality by central reader that may, in the opinion of the Investigator, interfere with study participation. Resting QTcF interval of ≥450 msec for males or ≥470 msec for females (by central reader).
20. BMI at Screening <25.0 or >50.0 kg/m^2.

Prior/Concomitant Therapy

21. Subject report of use of medications historically associated with secondary NAFLD for more than 2 consecutive weeks in the 12 months prior to screening (e.g., amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens or anabolic steroids at doses greater than those used for hormone replacement, valproic acid, and other medications with known hepatotoxicity). Inhaled corticosteroids are allowed.
22. Any prior exposure to a FGF21 analog (e.g., including but not limited to pegozafermin, LY2405319, LY3025876, BMS986036, BMS986171, PF05231023, PF-06645849, AKR-001) or FGFR1 activating product, if known.

23. Any investigational drug small molecule (new chemical entity) within 30 days and large molecule (biologics) within 90 days, or 5 half-lives, whichever is longer, prior to Day 1, if known.
24. Subjects taking vitamin E (> 400 IU/day) must be on a stable dose for at least 6 months prior to screening.

Prior/Concurrent Clinical Study Experience

25. Currently participating in or have participated in a study of an investigational agent or has used an investigational device within 30 days prior to the first dose of IP. Note: study participants will not be allowed to participate in other interventional trials for FGF21 analog (e.g., including but not limited to pegozafermin, LY2405319, LY3025876, BMS986036, BMS986171, PF05231023, PF-06645849, AKR 001) or FGFR1 activating products, during the immunogenicity follow up period.

Other Exclusions

26. Inability to undergo a liver biopsy safely for any reason.
27. Subject who cannot undergo MRI for any reason (e.g., contraindication, claustrophobia not controlled by anxiolytic, excessive weight or body size for MRI machine).
28. Subject who cannot fast for study procedures for any reason. Specifically, subjects with T2DM who have a history of clinically significant, symptomatic hypoglycemia or past issues with fasting will be excluded. Subjects with T2DM may need to consult their treating physician about the optimal timing to take their medications to enable them to fast safely for study procedures.
29. Any abnormality of the skin or abdominal wall that would impede SC administration to the abdominal area.
30. Known hypersensitivity to the components of the IP (refer to pegozafermin IB), or history of a severe hypersensitivity reaction that, in the opinion of the Investigator, might place the subject at risk to receive IP.
31. Pregnant or breastfeeding or planning to become pregnant or breastfeed while enrolled in the study or within 30 days after last dose of IP.
32. An employee of the investigational center or has a family member who is involved with the conduct of this study.
33. Any other clinically significant findings (including incidental findings during Screening), disorders or prior therapy that, in the opinion of the Investigator or Medical Monitor (or designee), would make the subject unsuitable for the study or unable to comply with the dosing and protocol requirements.

5.3. Lifestyle Considerations

At each visit the study staff should discuss diet and exercise, with the goal of lifestyle stabilization during the study.

To ensure standardization, all sites should provide the same guidance. Subjects should be encouraged to limit energy intake from total fats and sugars, increase consumption of fruit and vegetables, as well as legumes, whole grains, and nuts, and engage in at least 150 minutes of moderate-intensity aerobic physical activity throughout the week, or do at least 75 minutes of vigorous-intensity aerobic physical activity throughout the week, or an equivalent combination of moderate- and vigorous-intensity activity. Strenuous exercises should be avoided for at least 48 hours prior to study visits. Note that per Exclusion Criterion 12, certain physical activities such as starting a new weight loss program, training for a marathon, or taking a concomitant weight loss medication may have a confounding treatment effect on study drug and are therefore exclusionary. The Medical Monitor (or designee) should be contacted if there are any questions regarding lifestyle considerations.

Subjects should also be encouraged to limit alcohol intake to less than approximately 14 drinks per week (≤ 2 drinks per day) for men and 7 drinks per week (≤ 1 drink per day) for women. This guidance should be documented in the source and electronic case report form (eCRF). Subjects will be instructed to maintain the diet and activity level for the duration of the study. As part of lifestyle counseling, subjects will be questioned about change in diet or physical activity.

5.4. Screen Failures and Rescreening

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently randomized. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

If **any of the laboratory eligibility criteria**, ECG, or vital sign criteria are not met during the initial screening, subjects will be allowed to retest at the Investigator's discretion. If a subject fails to meet that laboratory eligibility criteria at the retests, they will remain ineligible. Investigator may also request a rerun (i.e., same sample to be analyzed again) at their discretion. In these instances, the 12-week screening window may be extended with Sponsor's (or designee) approval.

Subjects who do not meet **all eligibility criteria** will be allowed to rescreen with Medical Monitor (or designee's) approval. Subjects who rescreen will be required to sign a new ICF. Rescreened subjects whose liver biopsy qualified at their original screening will not repeat the biopsy during the rescreening period if the biopsy is still within the 6-month eligibility period allowed for historical biopsies. Other imaging assessments, such as MRI and Fibroscan, do not need to be repeated for rescreened subjects if those assessments are within the 3-month eligibility period respectively allowed for those assessments. Lab assessments will be repeated for rescreened subjects.

6. INVESTIGATIONAL PRODUCT

IP is defined as pegozafermin or matching placebo, intended to be administered to a study subject according to the study protocol.

6.1. Investigational Product Administered

IP will be administered SC to the abdomen region by qualified personnel in the clinic or in the subject's home, or by the subject/subject's caregiver following required training on IP administration. Some IP administrations will occur at the subject's home.

ARM	**Active– liquid formulation**	**Placebo (control)**
Investigational product	pegozafermin	Matching placebo
Type	Biologic	Chemical solution
Dose Formulation	Each 1.1 mL vial contains [redacted] mg of pegozafermin in Tris buffer solution (containing [redacted] at a concentration of [redacted] mg/mL	Each 1.1 mL vial contains Tris buffer solution (containing [redacted]
Unit Dose Strength(s)	mg	Not applicable
Dosage Level(s) [a]	QW 15 mg 30 mg	QW Matching placebo will be injected QW.
	Q2W 44 mg	Q2W Matching placebo will be injected Q2W.
Route of Administration	SC injection	SC injection
Administration Instructions	SC injection of the IP will be performed by qualified site staff when the subject attends Day 1 visit. Subjects and/or caregivers may also be trained on self-administration and thereafter self-administer the IP with staff witnessing on Day 1. Further doses will be administered at home by subject, subject's caregiver, or home healthcare services. Details on administration at home, including training requirements, are provided in the Home Administration Instructions or Pharmacy Manual.	
Sourcing	Provided centrally by the Sponsor	Provided centrally by the Sponsor
Packaging and Labeling	IP (pegozafermin and placebo) is supplied as a sterile, preservative-free, liquid formulation in a single-use Type 1 clear glass vial for SC injection. Each vial will be labeled per country requirement.	

[a] The actual doses will be ±5% the mg dose due to technical considerations related to drug withdrawal from the vials into the syringes for injection. This difference is considered negligible for subject exposure.

6.2. Administration Instructions

IP will be administered SC to the abdomen by trained and qualified study personnel/ site staff at clinic visit(s). IP will be administered by healthcare professional or trained subject's caregiver for home administration. IP can also be self-administered once a subject is appropriately trained. Details on self-administration at home, including training requirements will be provided in the Pharmacy Manual. Home healthcare services may be utilized for any subject requiring assistance with IP administration.

Injections will be administered in the abdominal area only. For additional doses in the same subject, the injection site should be rotated within the abdominal area by at least 2 inches. Injections should be at least 2 inches from the umbilicus (belly button) and should avoid scars or tattoos. After administration of the IP, the subject should remain resting for approximately 15 minutes.

6.3. Preparation/Handling/Storage/Accountability

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for any IP received and any discrepancies are reported and resolved before use of the IP.

Any IP stored on site, must be kept in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

The Investigator, institution, or the head of the medical institution (where applicable) is responsible for IP accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

Only subjects enrolled in the study may receive IP. Administration of IP may be performed by authorized site staff, home healthcare professionals, or study subjects (or their caregiver) who have been appropriately trained.

Further guidance and information for IP preparation and administration and the final disposition of unused IP is provided in the Pharmacy Manual.

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.

Details on preparation, handling, storage, and accountability for at-home visits are provided in the Home Administration Instructions or Pharmacy Manual.

6.4. Measures to Minimize Bias: Randomization and Blinding

6.4.1. Randomization and Stratification

Main study: All subjects will be centrally randomized 16:8:6:24:15 to Placebo QW, Placebo Q2W, pegozafermin 15 mg QW, 30 mg QW and 44 mg Q2W, respectively. The randomization will be stratified by T2DM status (yes vs. no) and fibrosis stage (F2 vs. F3). For the purposes of stratification, subjects will be considered to be T2DM status positive if any of the following

criteria are met: current medical history of T2DM, current use of anti-glycemic medication(s) for T2DM, or screening laboratory values of ≥6.5% for HbA1c or ≥126 mg/dL for fasting plasma glucose. The proportion of subjects enrolled with MRI-PDFF <8% may be limited at the Sponsor's discretion.

In the Extension study, subjects randomized to placebo QW in the Main study will be re-randomized 1:1 to pegozafermin 30 mg QW (n=21) or placebo (n=21).

All subjects will be centrally assigned to randomized IP using an Interactive Response Technology (IRT).

6.4.2. Assignment of Subject Number and Investigational Product

At the Screening visit, each subject will have a unique subject number assigned for subject identification in the study. Subjects who are rescreened will retain the original identification number; a new ID will not be assigned.

IP will be dispensed at the study visits as summarized in the SoA. The IP kit number(s) will be assigned by the IRT system upon obtaining the subject's randomized treatment group.

For subsequent visits when IP is dispensed, the IRT system will assign IP kits based on the subject's randomized treatment group in the Main study and the Extension study accordingly.

6.4.3. Blinding

Subjects, 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, MRI, PK and 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 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 Monitors. A formal unblinding plan will be established prior to the unblinding for Week 24.

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, and manage the unblinded data, manage IP inventory, manage expedited reporting of suspected unexpected serious adverse events (SUSARs), and who conduct unblinded analyses for the DMC. DMC members will have access to unblinded data for the purposes of reviewing unblinded overall safety results (Section 9.8).

6.4.4. Unblinding of an Individual Subject

The IRT system will be programmed with blind-breaking instructions. In case of a medical emergency, the Investigator has the sole responsibility for determining if unblinding of a

subject's randomized treatment is warranted. Subject safety must always be the first consideration in making such a determination. If the Investigator decides that unblinding is warranted, the Investigator, when possible, should make efforts to contact the Medical Monitor (or designee) to discuss unblinding a subject's treatment assignment before doing so, unless this could delay emergency medical treatment of the subject. If a subject's treatment assignment is unblinded, the Sponsor must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation and IRT.

Appropriate personnel at the Sponsor (or designee) will unblind SUSARs for the purpose of regulatory reporting. The Sponsor (or designee) will submit SUSARs to Regulatory Agencies in blinded or unblinded fashion according to local law. The Sponsor (or designee) will submit SUSARs to Investigators in a blinded fashion.

Appropriate personnel at the Sponsor (or designee) will have access to unblinded individual subject treatment assignments for the purposes of study-required activities including management of IP inventory, production of summary tables and figures for DMC review, and performance of bioanalytical analysis of PK concentrations. These personnel will not be involved in data collection or final analysis of safety and efficacy results. Subjects, investigators, and other site personnel who are directly involved in the conduct of the study, collection of the data, and analysis of the final safety and efficacy results will remain blinded to treatment assignments until after the completion of the study and the database has been locked.

6.5. Investigational Product Compliance

When subjects are dosed at the site, they will receive IP directly from the Investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents and recorded in the eCRF. The dose of IP and study subject identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the IP.

Details on IP compliance at remote/home visits are provided in the Home Administration Instructions or Pharmacy Manual. The allowed time window for dosing is ±2 days. If a subject wishes to change their dosing schedule (e.g., to a different day of the week), the Investigator should discuss with the Sponsor.

6.5.1. Guidance for Missed Dose(s)

The IP 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. If more than 3 days (72 hours) have elapsed from the scheduled dosing for either QW or Q2W, subjects will be instructed to skip the missed dose. The Medical Monitor (or designee) should be contacted if there are any questions on missed doses.

6.6. Treatment of Overdose

There is no experience with overdose of pegozafermin in humans. In Study TV47948-SAD-10122, single doses of pegozafermin at doses up to 78 mg were safe and well tolerated in healthy subjects.

For this study, any dose of IP greater than the highest protocol-specified dose will be considered an overdose.

The Sponsor does not recommend specific treatment for an overdose.

In the event of an overdose, the Investigator should be notified by the subject and:

- Contact the Medical Monitor (or designee) immediately.
- Closely monitor the subject for any AEs and laboratory abnormalities for at least 30 days.
- Obtain a serum sample for PK analysis within 2 days from the date of the overdose if requested by the Medical Monitor (or designee) (determined on a case-by-case basis).
- Document the quantity of the excess dose as well as the occurrence of the overdose in the eCRF.

6.7. Concomitant Therapy

Any medication (including over-the-counter or prescription medicines, vitamins, and herbal supplements) or vaccine that the subject is receiving at the time of enrollment or receives during the study must be recorded along with at least the following information:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency and route of administration

It has previously been reported that NAFLD is associated with reduced CYP3A drug metabolism activity in vivo and hepatic CYP3A4 expression in humans (Woolsey, 2015). In addition, based on the work by Woolsey et al it is suggested that exogenously administered FGF21 may decrease hepatic CYP3A4 activity (Woolsey, 2016). The underlying mechanism has not been fully elucidated.

Subjects receiving concomitant administration of CYP3A4 substrates with narrow therapeutic index should be closely monitored for any potentially related safety events, and dose adjustment of the CYP3A4 substrate should be considered as needed.

Short-term use of sliding scale insulin therapy during a medical procedure or hospitalization (<5 days) for subjects with T2DM may be performed as needed.

The Medical Monitor (or designee) should be contacted if there are any questions regarding concomitant or prior therapy.

6.7.1. Prohibited Medications/Therapies

Prohibited therapies include:

- Bariatric surgery
- Elective bone surgery for any bone disorders
- Medications historically associated with secondary NAFLD requiring >2 weeks of pharmacologic dosing . These include amiodarone, methotrexate, glucocorticoids,

tetracyclines, tamoxifen, estrogens or anabolic steroids at doses greater than those used for hormone replacement.

- Other hepatotoxic agents including but not limited to, nefazodone, isoniazid, valproate, and carbamazepine.
- The TNF (tumor necrosis factor) antagonists, adalimumab, etanercept and certolizumab, infliximab or sulfasalazine are prohibited during the Main Study but may be considered for managing emerging AEs in the Extension Study.
  - Short-term oral steroid bursts with/without taper as well as topical and inhaled corticosteroids are allowed.
- Antidiabetic medications: Thiazolidinediones (e.g., pioglitazone) are prohibited throughout study to minimize risk of confounding effect with pegozafermin treatment. Dose adjustments or switching from one medication to another within the same drug class are allowed after randomization for DPP-IV inhibitors ("gliptins"), SGLT2 inhibitors ("gliflozins") and GLP-1 agonists ("glutides", including semaglutide [Ozempic]); However, initiation of new DPP-IV inhibitors, SGLT2 inhibitors, or GLP-1 agonists is not allowed during screening and the Main Study (e.g., switching from a DPP-IV inhibitor to a GLP-1 agonist is not allowed, but switching from one DPP-IV inhibitor to another DPP-IV inhibitor is allowed). Initiation of these agents for uncontrolled hyperglycemia (but not for weight loss) may be allowed during the Extension Study with the Medical Monitor's approval.
- Vitamin E (> 400 IU/day) should not be initiated, or dose increased during the study.
- Any investigational product, FGF21 analogs (e.g., LY2405319, LY3025876, BMS-986036, BMS-986171, PF-05231023, AKR-001, pegozafermin), or FGFR1-activating products. Note: study participants will not be allowed to participate in other interventional trials for FGF21 analog or FGFR1 activating products, during the immunogenicity follow up period.
- Weight loss medications (including GLP-1 agonists specifically for weight loss) should not be initiated during the study.

The Investigator should discuss any questions regarding allowed/prohibited medications with the Medical Monitor (or designee). The final decision on any supportive therapy rests with the Investigator. However, the decision to continue the subject on IP requires the mutual agreement of the Investigator and the Sponsor.

6.8. Intervention after the End of Study

No additional intervention is planned beyond the end of the study.

7. DISCONTINUATION OF IP AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1. Discontinuation of Investigational Product and Subject Withdrawal from the Study

In some instances, it may be necessary for a subject to permanently discontinue IP. Temporary IP interruption may be allowed if Investigator and Medical Monitor (or designee) assess that IP rechallenge is safe and appropriate.

Permanent discontinuation of IP at any time during the study does not mean withdrawal from the study, and the subject will be encouraged to remain in the study, complete the Early Termination (ET) visit at the time of IP discontinuation and continue to complete remaining study period during the Main Study or the Extension Study as appropriate (i.e., subjects who ET before Week 24 may complete study visits through Week 24 and subjects who ET after Week 24 may complete visits through Week 48). Procedures for the remaining visits will include assessment of AEs and updating concomitant medications. Subjects who permanently discontinue IP at or after Week 16 and before Week 24 of the Main study will be requested to provide a liver biopsy at ET visit. (Section 1.3).

Subjects who experience clinically significant TEAEs that are assessed as a potential risk to subject safety will be discontinued from IP and undergo an ET visit as specified in the SoA. The decision to discontinue IP will be made by the Investigator and should be discussed with the Medical Monitor (or designee). If any subject experiences a Grade 3 TEAE that is considered related to IP, the Investigator should discuss treatment discontinuation with the Medical Monitor (or designee).

Subjects have the right to withdraw from the study at any time and for any reason without prejudice to their future medical care by the Investigator or at the institution. The reason for subject withdrawal from the study will be recorded in the eCRF. At the time of withdrawal from the study, the ET visit should be conducted as shown in the SoA.

A subject may discontinue IP or withdraw from the study for the following reasons:

- Adverse event
- Death
- Lack of efficacy
- Lost to follow-up
- Non-compliance with study drug
- Physician decision
- Pregnancy
- Protocol deviation
- Site terminated by Sponsor
- Study terminated by Sponsor

- Withdrawal by subject
- Randomized by mistake
- Evidence of hepatic decompensation including ascites, hepatic encephalopathy, splenomegaly, or variceal bleeding as assessed by the Investigator (for F4 subjects only).
- MELD Na+ score >12 (for F4 subjects only)

For liver chemistry abnormality actions and follow-up assessments, refer to Section 7.1.1.

Pregnancy is a mandatory criterion for permanent discontinuation of IP (Section 7.1.2).

7.1.1. Monitoring and Discontinuation for Suspected Drug-induced Liver Injury (DILI)

Liver chemistry will be evaluated as specified in the SoA (Section 1.3).

Per FDA recommendations, the following criteria for elevations in liver transaminases or bilirubin will be used for closely monitoring, discontinuing, or temporarily interrupting IP.

Definition of baseline ALT and AST values

Baseline value is defined as an average of ALT and AST values performed during Screening and the Baseline (Day 1) visit, as follows:

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)

ALT/AST Screening Assessments			Day 1 ALT/AST Assessment	Baseline Value
Assessment 1	**Assessment 2**	**Assessment 3 (if applicable)**		
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**

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase

New transaminase elevations in subjects with baseline value within the normal range:

For new elevations in transaminases to greater than 2x ULN, repeat measurement should be performed within 48-72 hours[10] of receipt of laboratory results. If elevations persist, subjects should be evaluated for other causes of transaminase elevations and with tests of hepatic function. If no other cause is identified, then the subjects need to be monitored closely (see below), and discontinuation of the IP should be considered.

IP should be discontinued, and the subject followed until resolution of symptoms or signs in the following situations:

- ALT or AST >8x ULN
- ALT or AST >5x ULN for more than 2 weeks
- ALT or AST >3x ULN and (TB[11] >2x ULN or INR >1.50[12])
- ALT or AST >3x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, jaundice (not attributable to Gilbert's syndrome), and/or eosinophilia (>5.0%)

New transaminase elevations in subjects with baseline ALT or AST > ULN

For new elevations in transaminases to greater than 2x baseline value or total bilirubin >1.5x ULN, repeat measurement should be performed within 48-72 hours[11] of receipt of laboratory results. If elevations persist, subjects should be evaluated for other causes of transaminase elevations and with tests of hepatic function. If no other cause is identified, then the subjects need to be monitored closely (see below), and discontinuation of the IP should be considered.

IP should be discontinued, and the subject followed until resolution of symptoms or signs in the following situations:

[10] In cases of isolated AST elevation to the indicated threshold, with a clear non-hepatic source for AST elevation (e.g., evidence of significant concurrent creatine phosphokinase elevation), decision regarding need to proceed with DILI work-up will be based on Investigator judgement.
[11] In subjects with Gilbert's syndrome, Direct bilirubin >2 x ULN.
[12] Subjects on anti-coagulation therapy must be assessed individually, as INR criterion will not apply.

Table 3: Discontinuation Criteria in Subjects with Abnormal Baseline ALT or AST Values

Baseline Value of ALT/AST	Criteria to Discontinue IP
<2× upper limit of normal (ULN)	if ALT or AST increases to >5× baseline value
≥2× ULN but <5× ULN	if ALT or AST increases to >3× baseline value
≥5× ULN	if ALT or AST increases to >2× baseline value
Other	if ALT or AST increase to >2× baseline value AND the increase is accompanied by a concomitant total bilirubin increase to >2× ULN OR the INR concomitantly increases by >0.2.
	if ALT or AST increase to >2× baseline value in the presence of signs and symptom(s) such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, jaundice (not attributable to Gilbert's syndrome) and/or eosinophil (>5%)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, normalized ratio; ULN, upper limit of normal

Close Monitoring for Suspected DILI:

- Repeating liver enzymes, serum bilirubin, hematology panel (for eosinophil count), and INR tests two or three times weekly. Frequency of repeat testing can decrease to once a week or less if abnormalities stabilize or the IP has been discontinued and the subject is asymptomatic.
- Obtaining a more detailed history of symptoms and prior or concurrent diseases.
- Obtaining a history of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
- Ruling out acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease.
- Obtaining a history of exposure to environmental chemical agents.
- Obtaining additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin).
- Considering gastroenterology or hepatology consultations.

Note: If a visit to the clinic is not feasible, laboratory testing can be performed by home health and sent to the central laboratory, or locally and the results should be promptly communicated to the Investigator site.

7.1.2. Monitoring and Study Continuation of Subjects with NASH Fibrosis Stage F1 or F4

The initial protocol allowed subjects to be included based on the biopsy read by one of the 2 expert pathologists. Version 3 Amendment #2 (March 11, 2022) changed the biopsy reading methodology to a central panel of 3 independent pathologists. This change in methodology

resulted in a change in fibrosis stage at baseline for some subjects (from F2 or F3 to either F1 or F4). These subjects and subjects evaluated as F4 during the study will be managed as follows:

1. Subjects with baseline fibrosis stage F1 as determined by a consensus of 3 central pathologists may continue all phases of the study and will be analyzed separately as an exploratory population.
2. Subjects with baseline fibrosis stage F4 (cirrhotic) as determined by a consensus panel of 3 central pathologists (as described above) will participate in all phases of the study as an exploratory population and will be analyzed separately provided:
    a. Their liver disease is considered well compensated as assessed by Child-Pugh Class A criteria (See Section 10.8), and
    b. There is no evidence of hepatic decompensation (i.e., hepatic encephalopathy, ascites, or bleeding varices) as assessed by the PI according to usual standard of care, and
    c. There is no evidence of hepatocellular carcinoma as assessed by local standard of care (e.g., ultrasound, CT scan, or MRI imaging methods and/or central AFP measurement). Central AFP measurement will be performed at every scheduled visit and may be done locally if applicable.
    d. Their MELD Na+ score is ≤ 12. The MELD Na+ score can be calculated by the Investigator, if applicable.
3. Subjects who complete the Main Study and have Week 24 biopsy results evaluated as F4 (cirrhotic) will complete all remaining study periods, provided they continue to meet the criteria in 2a-d above.
4. Consistent with these requirements, subjects who are F4 (cirrhotic) and have evidence of decompensation at any time during the study will be discontinued per Section 7.1 discontinuation criteria above.

7.1.3. Pregnancy

A female subject must permanently discontinue IP if she becomes pregnant. See Section 10.4 and Section 8.3.5 for additional details. If a male subject's partner becomes pregnant, the male subject must agree to use condoms with spermicide to prevent potential fetal exposure.

See the SoA (Section 1.3) for data to be collected at the time of IP discontinuation (ET visit).

7.2. Lost to Follow-up

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site must attempt to contact the subject and reschedule the missed visit/procedures as soon as possible and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to or should continue in the study.

- Before a subject is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study (and be labelled as lost to follow-up).
- Discontinuation of specific sites or of the study as a whole are handled as part of Appendix 1 (Section 10.1.8).

8. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoA (Section 1.3).
- If multiple assessments are scheduled at the same timepoint, it is recommended that procedures be performed in the following sequence: PROs, 12-lead ECGs, vital signs, physical examination, sample collection for laboratory and PD biomarker tests, and sample collection for PK.
- The Medical Monitor (or designee) should be notified about immediate safety concerns upon occurrence or awareness, as soon as possible. Decisions if the subject should continue or discontinue IP will be per Investigator's discretion, in consultation with the Medical Monitor (or designee), if possible.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- Any situation at the site level with potential impact on subject safety or study conduct, including situations related to COVID-19 infection or control measures (Appendix 6, Section 10.6), should be discussed with the Sponsor immediately upon occurrence or awareness to determine potential impact on study subject/s or study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The Investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the subject's routine clinical management (e.g., blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the timeframe defined in the SoA.

8.1. Efficacy Assessments

Laboratory-associated PD and biomarker assessments are described in Section 8.5.

8.1.1. Liver Biopsy and Scoring

Liver biopsy remains the gold standard for diagnosing NASH as well as for establishing the degree of liver fibrosis as it can reliably assess the various histopathological patterns characteristic of NASH (Andronescu, 2018; Arab, 2018; Bedossa, 2018; Gunn, 2018). In addition, histopathology scores are used to assess extent of fibrosis before and after treatment. The fibrosis stage ranges from F0 (without fibrosis) to F4 (cirrhosis) per NASH CRN system. Currently, liver biopsy is required by regulatory authorities as a surrogate endpoint to assess drug efficacy in Phase 3 clinical studies.

Paired liver-biopsy will be performed percutaneously (preferred method) per institution standard, at Screening (a recent biopsy within 6 months prior to screening will be acceptable if available for central read evaluation in lieu of screening biopsy) and Week 24 (window of -7 days to +14 days) (as specified in the SoA, Section 1.3). If biopsy is not performed by Week 26 for subjects in QW dose groups, further doses should be held until discussion with Sponsor. Subjects who

permanently discontinue IP at or after Week 16 and before Week 24 of the Main study will also be requested to provide a liver biopsy at ET visit. For any other scenarios of IP discontinuation or withdrawal from the study, consult the Medical Monitor (or designee) regarding biopsy collection.

Biopsy assessment for eligibility and endpoints will be based on NASH CRN. The NASH CRN adopted the system validated by Kleiner et al. (Kleiner, 2005). 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

NASH CRN Fibrosis Staging System	
Stage	**Fibrosis**
Stage 0	None
Stage 1	Perisinusoidal or periportal
Stage 1A	Mild, zone 3, perisinusoidal
Stage 1B	Moderate, zone 3, perisinusoidal
Stage 1C	Portal/periportal
Stage 2	Perisinusoidal and portal/periportal
Stage 3	Bridging fibrosis
Stage 4	Cirrhosis

Transjugular or other biopsy methods may be utilized with Medical Monitor (or designee) approval if the Investigator deems it to be a safer option for an individual subject, and if the site has extensive experience with obtaining biopsies of adequate size using the technique. Ideally, liver biopsies should be done after MRI-PDFF.

Details on the biopsy procedure, preparation of slides and central read are provided in the Biopsy Manual. The procedure will be performed by an experienced hepatologist or radiologist. Biopsies will be subject to central read by liver pathologists who are blinded to the treatment group assignment.

In addition to the central read, liver histology will also be quantitatively assessed by an AI-based machine-read analysis.

8.1.2. Magnetic Resonance Imaging – Whole Liver Proton Density Fat Fraction (MRI-PDFF)

MRI-PDFF is as a noninvasive, quantitative, and accurate measure of liver fat content (imaging-based biomarker) to assess treatment response in NASH clinical studies (Caussy, 2018). In this study, the MRI-PDFF procedure is used to assess secondary and exploratory endpoints and will be performed at timepoints specified in the SoA.

PDFF

The MRI-PDFF is determined using a 6-echo gradient echo pulse sequence covering the liver in the axial plane. Analysis is performed by semi-automatic contouring of the liver in every slice avoiding major vessels and bile ducts. The method applies multi-peak lipid spectral models and simultaneous quantification and correction for T2. The liver fat value (PDFF) is the pooled median value of all voxels in the identified volume of interest. For more information, refer to the Imaging Manual.

At Screening, MRI-PDFF should ideally be performed prior to the liver biopsy. A historical MRI-PDFF performed within the last 3 months prior to screening may be acceptable if the images are available and evaluable by the central imaging vendor. The proportion of subjects enrolled with MRI-PDFF <8% may be limited at the Sponsor's discretion.

Iron-corrected T1 Mapping (cT1)

Applicable only at sites capable of conducting cT1 imaging.

cT1 imaging is an MRI-derived measurement of iron-corrected T1 mapping (cT1). It is a non-invasive, quantitative and accurate biomarker which correlates with ballooning (Pavlides, 2017; Eddowes, 2018), fibrosis (Banerjee, 2014; Pavlides, 2017; Tunnicliffe, 2017; McDonald, 2018), and NAS (Eddowes, 2018) and has also been shown to predict clinical outcomes (Pavlides, 2016). T1 mapping measures longitudinal relaxation time, which can be used as an indication of regional tissue water content.

cT1 is measured by using the LMS MOLLI sequence, which is a multi-slice, multi-breathhold, cardiac-gated acquisition performed at end-expiration. It is determined using a five-slice transverse acquisition with one breathhold of approximately 10 seconds per slice. This protocol uses a ShMOLLI acquisition on GE and Siemens scanners, and a MOLLI acquisition on Philips.

At Screening, a historical cT1 imaging 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 and Spleen Volume

A dedicated axial 3 dimensions (3D) T1-weighted spoiled gradient echo scan with or without fat suppression will be positioned to cover the entire liver and spleen. Analysis will be done using a semiautomated software to delineate the outer borders of the liver and the liver volume will be calculated in liters.

At Screening, a historical liver and spleen volume 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.

Pancreatic Fat

An IDEAL (iterative decomposition of water and fat with echo asymmetry and least-squares estimation) multi-echo-gradient echo sequence with 6-point gradient echo will be performed in the axial plane cantered on the pancreas covering 10 cm of the organ in the feet-head direction. The pancreatic fat will be reported in % of proton density fat fraction.

At Screening assessment, a historical 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.

8.1.3. Transient Elastography (FibroScan®) kPa and CAP scores

FibroScan is a validated non-invasive specialized ultrasound assessment of liver fibrosis and steatosis (Wong, 2013). Fibrosis and liver stiffness are measured in kilopascals (kPa).

In this study, both VCTE and CAP will be measured:

- VCTE has a high negative predictive value to rule out advanced fibrosis; positive predictive value when it indicates advanced fibrosis is actually inadequate and insufficient to diagnose cirrhosis. VCTE is mostly to differentiate advanced fibrosis vs. absence of advanced fibrosis and has limited accuracy in differentiating fibrosis stages that are not at the ends of the spectrum.
- The CAP score is a measurement of fatty change in the liver and is measured in decibels per meter (dB/m).

Specifics for performance of the FibroScan will be in accordance with manufacturer's standards and instructions or manuals.

FibroScan assessments will be performed at timepoints specified in the SoA. At Screening, a historical FibroScan performed within the last 3 months prior to screening may be acceptable.

8.1.4. Anthropometric Measurements

The following anthropometric measurements will be collected during the study as specified in the SoA:

- Body weight
- BMI (auto-calculated)
- Waist circumference
- Hip circumference
- Waist/hip ratio (auto-calculated)

Further information will be provided in the Investigator's site file.

8.1.5. Patient Reported Outcome

All PROs should be completed prior to dosing.

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

CLDQ NAFLD-NASH is a self-reported 36-item quality of life questionnaire composed of 6 domains (abdominal symptoms, activity/energy, emotional health, fatigue, systemic symptoms and worry). CLDQ-NAFLD/NASH was shown to be reliable and was validated in 1667 patients with biopsy-confirmed NASH enrolled in 2 Phase 3 studies (Younossi, 2019b).

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

WPAI-NASH is a well-validated, self-administered instrument measuring impairment of work and non-work activities over the prior 7 days in employed subjects. 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). Higher scores indicate a higher absence from work and impairment (Reilly, 1993). This instrument was shown to be valid and reproducible including in subjects with NASH (Balp, 2019).

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

The EQ-5D-5L was introduced by the EuroQoL Group in 2009 to improve the instrument's sensitivity and to reduce ceiling effects, as compared to the EQ-5D-3L, including in subjects with chronic hepatic disease (Janssen, 2013; Scalone, 2013). The EQ-5D-5L essentially consists of 2 pages: the EQ-5D descriptive system and the EQ VAS.

The descriptive system comprises 5 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. The subject is asked to indicate his/her health state by ticking the box next to the most appropriate statement in each of the 5 dimensions. This decision results in a 1-digit number that expresses the level selected for that dimension. The digits for the 5 dimensions can be combined into a 5-digit number that describes the subject's health state.

The EQ VAS records the subject's self-rated health on a vertical VAS, where the endpoints are labelled 'The best health you can imagine' and 'The worst health you can imagine'. The VAS can be used as a quantitative measure of health outcome that reflect the subject's own judgement.

The EQ-5D-5L utility score can range between 0 (death) and 1 (perfect health), while VAS score ranges from 0 (worst imaginable health) to 100 (best imaginable health).

8.1.5.4. Liver-related Pain and Discomfort Questionnaire

NAFLD/NASH may be associated with right upper quadrant pain or discomfort. To evaluate the effect of treatment with pegozafermin on liver-related pain and discomfort, a targeted questionnaire with a graphic that clearly indicates the right upper quadrant, where this pain is typically experienced, will be assessed. These questions will be similar to abdominal pain and discomfort items in the CLDQ-NAFLD-NASH questionnaire.

8.1.5.5. The Appetite Sensations Visual Analog Scale (VAS)

VAS measures the intensity or magnitude of sensations and subjective feelings and the relative strength of attitudes and opinions about specific stimuli, on a scale of 0 to 10 (Duncan, 1989). VAS scores are reliable for appetite research and do not seem to be influenced by prior diet standardization (Flint, 2000; Parker, 2004). For this study, VAS will be used to record hunger; satiety; fullness; prospective food consumption; and desire to eat something fatty, salty, sweet or savory.

8.2. Safety Assessments

Safety assessments include AEs (either reported by the subject or observed by the Investigator), concomitant medication use, physical examination, ECG, vital signs, and laboratory assessments. Planned timepoints for all safety assessments are provided in the SoA (Section 1.3).

8.2.1. Physical Examinations/Assessment

- A complete physical examination will include, at a minimum, assessments of the skin, respiratory, cardiovascular system, abdomen (liver and spleen) and a neurological exam. Height (only collected at Screening) will also be measured and recorded.
- A limited physical assessment will be conducted if clinically indicated and may be performed by home health nurse at remote visits; this should include at a minimum, assessments of the skin (inspection of injection sites), respiratory, cardiovascular system, and abdomen, and any other pertinent system based on prior findings or complaints or subject complaint.

8.2.2. Vital Signs

- Vital signs include blood pressure, pulse, body temperature, and respiratory rate.
- Planned timepoints for vital signs are provided in the SoA.
- 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 and repeat measurements should be performed according to local practice.
- 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, which should be performed according to local practice.

8.2.3. Electrocardiograms

- 12-lead ECG will be recorded as single bedside measurements using an ECG machine that automatically calculates the heart rate and measures PR, QRS, and QT (QTcF) intervals.
- Subject to be resting for at least 2 minutes prior to ECG.
- A central reviewer will be used; instructions will be provided in the imaging manual. ECG tracing should be provided to the central reader within 72 hour of assessment.

8.2.4. Bone Mass Density

BMD will be assessed by DXA, a standard reference test for evaluation of bone loss.

8.2.5. Clinical Safety Laboratory Assessments

See Appendix 2 (Section 10.2) for the list of clinical laboratory tests to be performed and the SoA (Section 1.3) for the timing and frequency. Laboratory tests should be performed under

fasting conditions (≥10 hours). Fasting includes food and all beverages except for non-mineral water. It is recommended that subjects abstain from consumption of alcoholic beverages for at least 24 hours before laboratory test assessments. It is recommended that subjects refrain from strenuous activities.

The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the subject's condition.

All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 28 days after the last dose of IP should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator.

If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified, and the Sponsor notified.

All protocol-required laboratory assessments, as defined in Appendix 2 (Section 10.2), must be conducted in accordance with the relevant study manual and the SoA (Section 1.3).

If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in subject management or are considered clinically significant by the Investigator (e.g., SAE or AE or skipping of dose), then the results must be recorded in the eCRF and the Sponsor should be notified.

8.2.5.1. Immunogenicity Assessments

Immunogenicity samples will be collected from all subjects at timepoints designated in the SoA (Section 1.3). Antibodies to pegozafermin as well as titer and binding specificity will be evaluated in serum samples collected from subjects treated with pegozafermin only.

For post-treatment 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 [redacted]. 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.

Samples for endogenous FGF21 level will be collected at baseline for all subjects, and at the immunogenicity follow-up visit(s). Endogenous FGF21 samples will be analyzed only in subjects with [redacted] to pegozafermin.

Serum samples will be screened for antibodies binding to pegozafermin and the titer of confirmed positive samples will be reported. Other analyses may be performed to verify the stability of antibodies to pegozafermin and/or further characterize the immunogenicity of pegozafermin. The detection and characterization of antibodies to pegozafermin will be performed using a validated assay method under the supervision of the Sponsor. Antibodies may be further characterized and/or evaluated for their ability to neutralize the activity of the IP(s). Samples may be stored for a maximum of 15 years (or according to local regulations) following

the last subject's last visit for the study at a facility selected by the Sponsor to enable further analysis of immune responses to pegozafermin.

8.2.5.2. Bone Marker Assessments

Plasma samples for potential future assessment of the bone markers CTX and P1NP will be obtained at the designated timepoints.

8.2.5.3. Cortisol Assessments

Saliva samples for measurement of night-time salivary cortisol (NSC) will be obtained at timepoints designated in the SoA (Section 1.3) to screen for hypercortisolism (Pappachan, 2017). The cortisol screening assessment is performed at screening to obtain baseline values and at subsequent visits to assess for hypercortisolism while on treatment. The 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.

The screening NSC assessment should be performed as early as possible during the screening period to allow sufficient time for samples to be resulted prior to randomization. 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. Subjects will not be deemed ineligible if screening NSC samples have been submitted but have not been resulted by Day 1 and may be randomized. Subjects with missing screening values will still have NSC assessments performed at the other scheduled visits as designated in the SOA.

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 criterion.

For on-treatment values that are >15 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 the repeat assessment is still > 15 nmol/L AND above the screening value, an additional screening test for hypercortisolism will be done (either overnight dexamethasone (1 mg) suppression test [DST] or a 24 h collection for urinary free cortisol [UFC]) level.

If a screening value is not available, and on-treatment value is >15 nmol/L, a repeat NSC should be performed and if the repeat assessment is still >15nmol/L, an additional test for hypercortisolism will be done (either a DST or a UFC).

The Medical Monitor (or designee) will provide guidance on the appropriate assessment based on the individual subject for subsequent test values that are also >15nmol/L based on the subject profile.

8.3. Adverse Events and Serious Adverse Events

The definitions of an AE and SAE can be found in Appendix 3 (Section 10.3).

AEs will be reported by the subject (or, when appropriate, by a caregiver, surrogate, or the subject's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following

up AEs that are serious, considered related to the IP or study procedures, or that caused the subject to discontinue the study or IP (see Section 7).

8.3.1. Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be as specified in the SoA (Section 1.3).

Table 4 below summarizes the different reporting periods for AEs, SAEs, and events to monitor. Events to monitor are defined in Section 10.3.3.

Table 4: Adverse Event Reporting Periods

Type of Event	Adverse Event	Serious Adverse Event	Events to monitor with IP
Reporting period	From consent until 28 days after last dose of IP	From consent until 28 days after the last dose of IP	From consent until 28 days after the last dose of IP
Reporting Timelines to the Sponsor	Entered into the clinical database on an ongoing basis	Within 24 hours	Within 24 hours

Note: For the immunogenicity follow-up period adverse events and serious adverse events will be collected at every visit until subject completes the last immunogenicity follow-up visit. Reporting timelines to the Sponsor for adverse events and serious adverse events remains the same as above.

All SAEs will be recorded and reported to the Sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Appendix 3 (Section 10.3). The Investigator will submit any updated SAE data to the Sponsor or designee within 24 hours of it being available.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event to be reasonably related to the IP or study participation, the Investigator must promptly notify the Sponsor or designee.

8.3.2. Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting SAE reports are provided in Appendix 3 (Section 10.3).

Care will be taken not to introduce bias when detecting AEs or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

8.3.3. Follow-up of AEs and SAEs

After the initial AE or SAE report, the Investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up (as defined in Section 7.2). Further information on follow-up procedures is given in Appendix 3 (Section 10.3).

8.3.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the Investigator to the Sponsor or designee of an SAE is essential so that legal obligations and ethical responsibilities to ensure the safety of subjects and the safety profile of IP under clinical investigation are met.
- The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety profile of IP under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs), and investigators.
- Investigator safety reports must be prepared for SUSARs according to local regulatory requirements and Sponsor policy and forwarded to investigators as necessary.
- An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

8.3.5. Pregnancy

- Details of all pregnancies in female subjects and of female partners of male subjects will be collected after the start of IP and until 90 days after last dose of IP.
- If a pregnancy is reported, the Investigator should inform the Sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 4 (Section 10.4).
- Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

8.4. Pharmacokinetics

Blood samples for analysis of pegozafermin serum levels will be collected predose at the visits designated in the SoA (Section 1.3).

Blood samples will be processed for collection of serum fractions for determination of pegozafermin serum concentrations. Serum samples will be shipped to the bioanalytical laboratory for analysis. PK samples collected from subjects during the period when they are administered placebo will not be analyzed.

8.5. Pharmacodynamics and Biomarkers

8.5.1. Pharmacodynamics

The following biomarkers/PD parameters will be evaluated at timepoints designated in the SoA (Section 1.3):

Laboratory Parameters

- Liver function tests: ALT, AST
- GGT
- ALP
- Pro-C3 (see Section 8.5.1.1)
- Adiponectin
- Triglycerides
- HDL-c
- Non-HDL-c
- LDL-c
- Total cholesterol
- HbA1c
- C-peptide
- Fasting glucose
- Fasting insulin

- Adipo-IR index (fasting free fatty acids x fasting insulin)
- High sensitivity C-reactive protein (hsCRP)
- ELF panel (Section 8.5.1.2)
- FIB-4 index (Section 8.5.1.4)
- AFP (F4 subjects only)
- MELD Na+ score (F4 subjects only)

Samples may be stored for a maximum of 15 years after the last subject's last visit for the study, at a facility selected by the Sponsor, to enable further analysis of biomarker responses to pegozafermin.

Residual blood samples will be stored for potential future analysis of biomarkers.

Additional information will be available in the study-specific laboratory manual.

8.5.1.1. N-terminal Propeptide of Type III Collagen (Pro-C3)

N-protease cleaved amino-terminal type 3 procollagen peptide (P3NP) neo-epitope (Pro-C3) is derived from the synthesis of type 3 collagen. Pro-C3 appears to correlate with liver fibrosis stage, fibrosis regression and response to treatment both as a single test and as part of algorithms (Nielsen, 2015; Daniels, 2018; Hansen, 2018).

8.5.1.2. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)

HOMA-IR is a method used to quantify insulin resistance based on the following formula(s) (Matthews, 1985):

Glucose in mmol/L

$$\text{HOMA-IR} = \frac{\text{Glucose} \times \text{Insulin}}{22.5}$$

Glucose in mg/dL

$$\text{HOMA-IR} = \frac{\text{Glucose} \times \text{Insulin}}{405}$$

HOMA-IR will be calculated by the central laboratory based on fasting glucose and insulin.

8.5.1.3. Enhanced Liver Fibrosis (ELF) Panel

The enhanced liver fibrosis (ELF) blood test has recently been recommended by the National Institute for Health and Care Excellence to test for advanced fibrosis in NAFLD. The ELF test involves calculating a score from the concentrations of serum biomarkers: tissue inhibitor of matrix metalloproteinases-1 (TIMP-1), P3NP, and hyaluronic acid (HA) (Lichtinghagen, 2013).

The ELF score may be calculated from TIMP-1, P3NP and HA measurements according to the following formula:

$$ELF\ Score = 2.278 + 0.851(\ln[HA]) + 0.751(\ln[P3NP]) + 0.394(\ln[TIMP\text{-}1])$$

8.5.1.4. FIB-4 Index

The FIB-4 is a simple non-invasive test for liver fibrosis that takes into account age, platelet count (PLT), AST, and ALT using the following formula:

$$age\ ([yr]) \times AST\ [U/L]) / ((PLT\ [10^9/L]) \times (ALT\ [U/L])^{1/2})$$

Subjects with a cut off of >3.25 have a positive predictive value of 65% for advanced fibrosis (Sterling, 2006).

8.5.1.5. Exploratory Biomarker Assessments

Samples for RNA/DNA, as well as plasma and serum samples, will be collected at designated timepoints from all subjects who consented from centers in which IRB/IEC approval is granted. These samples will be taken for potential future exploratory assessments, to increase understanding of pegozafermin biological activity and to identify potential existing and/or emerging biomarkers.

8.6. Pharmacogenomics Assessments

Pharmacogenomics (PGx) analyses may be performed on any bio-sample from subjects who have consented for PGx sampling. Subject confidentiality will be maintained. Please also refer to Appendix 5 (Section 10.5).

Suitable bio-samples should be collected from all subjects, where permitted by law and local authorities. Subjects who are prohibited from participating in the PGx research by law and/or local authorities and subjects providing a written (documented) opt-out may still participate in the study.

A single blood sample will be collected from all enrolled subjects for possible future genetic analyses related to pegozafermin, including associations of DNA variations with clinical treatment responses to pegozafermin (e.g., PK, tolerability, and safety features or disease susceptibility and severity features). Pharmacogenomic assessment may include a sequencing of the whole genome, if required.

9. STATISTICAL CONSIDERATIONS

This section describes the statistical analysis and intended methodology as foreseen at the time of planning the study. Changes, additions, and further details about the analyses will be described in the statistical analysis plan (SAP). Any subsequent additional analyses or changes to analyses that will be fully disclosed in the clinical study report. Summaries and analyses will be presented by dose/treatment group. Before database lock, final statistical and PK analysis plans containing full details of all planned analyses will be produced. The analyses presented here represent an outline of the intended methodology; Analyses specified in the SAP will take precedence over those described herein. All clinical data will be summarized using descriptive statistics: number (n), mean, standard deviation (SD), median, minimum, and maximum for continuous measurements, and counts and percentages for categorical measurements.

9.1. Statistical Hypotheses

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.

9.1.1. 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.
- 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 the end of the treatment period, 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, as detailed in the SAP.

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 relevant patient baseline covariates 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 relevant patient baseline covariates 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 ICE3. 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.

9.2. Sample Size Determination

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

9.3. Populations for Analyses

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.

Population	Description
MRI-PDFF Analysis Set	All subjects in the Full Analysis Set who have a baseline and at least one follow up MRI-PDFF assessment.

9.4. Statistical Analyses

The SAP will be developed and finalized before the database is unblinded for the Primary Analysis and will describe the subject populations to be included in the analyses, and procedures for accounting for missing, unused, and spurious data.

In general, summaries and analyses will be presented by treatment group. Descriptive statistics will be presented for demographics and baseline characteristics, efficacy and safety endpoints, and PK parameters, when appropriate.

For analysis purpose, placebo QW and placebo Q2W will be pooled. Supportive analyses of each treatment QW arm vs. placebo QW arm and 44 mg Q2W arm vs. placebo Q2W arm will also be performed. All statistical tests will be 2-sided and tested at a statistically significant level of 0.05. Unadjusted p-values will be reported for all efficacy comparisons. Confidence intervals (CI) will be 2-sided 95%, unless stated otherwise.

9.4.1. Efficacy Analyses

The primary analyses of the primary endpoints will be performed using the FAS subjects with confirmed fibrosis stage F2 or F3 and NAS ≥4 at baseline per a panel of 3 pathologists. To evaluate the effect of pegozafermin on liver histology, a stratified Cochran-Mantel-Haenszel (CMH) test will be used to compare the differences in proportions of subjects who met histological responder criteria at Week 24 between each pegozafermin group and placebo, adjusting for the stratification factors. The point estimates and 95% CI for the differences in proportion will be calculated. Different sensitivity analyses to assess the impact of intercurrent events as described in Section 9.1.1 will also be explored. The details of how to handle intercurrent events, missing 24-week histology data, and the sensitivity analyses will be described in the SAP.

Other binary efficacy endpoints will be analyzed with the methods analogous to those used for the histological endpoint. Other continuous efficacy endpoints will be analyzed by analysis of variance (ANCOVA) (for single post-baseline measurement), or by a mixed model repeated measures (MMRM) method. The model will include treatment group, week and treatment-by-week interactions as main effects and baseline measurement and stratifications as covariates. 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. If the data shows strong evidence of a violation of the model assumptions, non-parametric methods will be considered.

9.4.2. Safety Analyses

All safety analyses will be performed on the Safety Analysis Set. Safety assessments will be based on AEs, laboratory values, concomitant medication use, ECG, and vital signs data. Summaries will be presented by treatment group.

Treatment duration and IP received will be summarized by treatment group. Subject incidence of TEAEs will be tabulated by Medical Dictionary for Regulatory Activities (MedDRA) system organ class, MedDRA preferred term, and treatment group. All AEs that started within the first dose of IP and the last dose of the IP+28 days or continuing AEs that worsen the grade post IP will be considered as TEAEs. For subjects who withdraw consent from the study, the end date of the TEAE reporting period will be the earlier of the withdrawal consent date and the date of the last dose of the IP + 28 days. All TEAEs, all treatment-related TEAEs, all treatment-emergent SAEs, and all treatment-emergent serious related AEs will be summarized.

The number (%) of subjects who fail to complete the study and the reason for discontinuation, and the number (%) of subjects who fail to complete the study due to related TEAEs will be summarized and listed.

Safety laboratory tests, concomitant medication use, vital signs, and ECG measures will be summarized by visits and by treatment group.

Results of immunogenicity assessment will be provided by ADA response (number and percent of positive ADA subjects) and immunogenicity profile (e.g., antibody titers, binding specificity), when appropriate.

The effect of ADA on serum pegozafermin concentrations may be evaluated.

9.4.3. PK Analyses

Serum concentration data will be listed by subject and summarized using descriptive statistics by nominal timepoint.

9.5. Interim Analyses

No interim analysis of the primary endpoint is planned.

9.6. Primary Analysis

The primary analyses will be conducted according to the SAP after all subjects have completed the Week 24 assessments or prematurely discontinued from the study.

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 a separate document. Subjects and investigators/site personnel will remain blinded for the extension safety study.

9.7. Final Analyses

After all subjects have completed the EOS visit and the data have been cleaned and finalized, the study blind will be broken and the final analysis of the data will be performed according to the SAP.

9.8. 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.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1. Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
  - Applicable International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines
  - Applicable laws and regulations
- The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.
- The Investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
  - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

10.1.2. Financial Disclosure

Investigators and sub-investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3. Informed Consent Process

- The Investigator or his or her representative will explain the nature of the study to the subject or his or her legally authorized representative and answer all questions regarding the study.
- Subjects must be informed that their participation is voluntary. Subjects will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the subject was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the subject or the subject's legally authorized representative.

Subjects who are rescreened are required to sign a new ICF.

10.1.4. Data Protection

- Subjects will be assigned a unique identifier by the Sponsor. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.
- The subject must be informed that his or her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject.
- The subject must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5. Dissemination of Clinical Study Data

A clinical study report will be developed by the Sponsor at completion of data analysis. This report will be a clinical and statistical integrated report, according to the ICH E3 guidelines.

Sponsor will register the study and post study results regardless of outcome on a publicly accessible website in accordance with the applicable laws and regulations.

10.1.6. Data Quality Assurance

- Electronic records and computerized systems used to capture clinical study data will be maintained and validated in accordance with ICH and FDA guidelines.

- All subject data relating to the study will be recorded on the eCRFs unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The Sponsor assumes accountability for actions delegated to other individuals (e.g., contract research organizations).
- Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements. It may become required that some or all monitoring activities will be halted or performed remotely due to the recent COVID-19 pandemic. In such a case, details of the changes made to the monitoring strategy will be described in the Monitoring Plan.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator per ICH-GCP and local regulations or institutional policies. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor, whether within the retention period or thereafter.

10.1.7. Source Documents

- Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.
- Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

- Source documents are original documents, data, and records from which the subject's eCRF data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

10.1.8. Study and Site Closure

The Sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The Investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given to the Sponsor in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the Investigator
- Discontinuation of further IP development

If the study is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the subject and should assure appropriate subject therapy and/or follow-up.

10.1.9. Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.
- The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with the International Committee of Medical Journal Editors authorship requirements.

10.2. Appendix 2: Clinical Laboratory Tests

- The clinical laboratory tests detailed in Table 5 will be performed by a central laboratory, except if noted otherwise, at timing/frequency detailed in the SoA (Section 1.3). Laboratory tests will be performed under fasting conditions (≥10 hours).
- Protocol-specific requirements for inclusion or exclusion of subjects are detailed in Section 5.
- Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.
- Investigators must document their review of each laboratory safety report.

Table 5: Protocol-Required Laboratory Assessments

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	

Urinalysis (spot urine)	
Basic Urinalysis (dipstick, including macroscopic appearance, bilirubin, blood, color, glucose, ketones, leukocyte esterase, nitrite, pH, protein, specific gravity, urobilinogen);	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.
Other Study-Specific Laboratory Assessments	
Pegozafermin (to be evaluated by bioanalytical laboratory) 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. 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	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)

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	

10.3. Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1. Definition of AE

AE Definition

- An AE is any untoward medical occurrence in a clinical study subject, temporally associated with the use of IP, whether or not considered related to the IP.
- NOTE: An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of IP.

Events Meeting the AE Definition

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (i.e., not related to progression of underlying disease). A clinically significant abnormality is a confirmed abnormality (by repeat testing) that is changed sufficiently from Baseline so that in the judgment of the Investigator a change in management is warranted. This alteration may include: monitoring the laboratory test further, initiating other diagnostic tests or procedures, changing ongoing treatment, or administering new treatment.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after IP administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either IP or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events NOT Meeting the AE Definition

1. Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the subject's condition.
2. The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition.
3. Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
4. Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
5. Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.3.2. Definition of Suspected and Unsuspected Adverse Reaction

Suspected adverse reactions are defined as:
• Any AE for which there is a reasonable possibility that the IP caused the AE. For the purposes of Sponsor regulatory safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the IP and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by an IP.
Unexpected AEs are defined as:
• AE which is not listed in the IB of the IP or is not listed at the specificity or severity that has been observed.

10.3.3. Definition of Events to Monitor

Sponsor-defined Events to Monitor for Pegozafermin:
Events to Monitor include immunogenicity and hypersensitivity reactions occurring after the first administration of study agent(s) in subjects participating in this clinical study. These events must be reported by the Investigator to the Sponsor within 24 hours and are to be considered serious (for regulatory reporting purposes) only if they meet the definition of an SAE. These events are to be reported on an SAE form.

10.3.4. Definition of SAE

If an event is not an AE per definition in Section 10.3.1, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence that, at any dose:
1. Results in death
2. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.
3. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an SAE. Treatment or observation on an emergency outpatient basis not resulting in hospital admission is not considered an SAE.
4. Results in persistent disability/incapacity • The term disability means a substantial disruption of a person's ability to conduct normal life functions. • This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma

An SAE is defined as any untoward medical occurrence that, at any dose:
(e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
5. Is a congenital anomaly/birth defect
6. Other situations: • Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. • Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.5. Recording and Follow-Up of AE and/or SAE

AE and SAE Recording
• When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event. • The Investigator will then record all relevant AE/SAE information in the eCRF. • It is not acceptable for the Investigator to send photocopies of the subject's medical records to the Medical Monitor (or designee) in lieu of completion of the AE/SAE eCRF page. • There may be instances when copies of medical records for certain cases are requested by the Medical Monitor (or designee). In this case, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to the Medical Monitor (or designee). • The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
Assessment of Intensity
The severity of each AE will be assessed at onset by a nurse and/or physician. When recording the outcome of the AE the maximum severity of the AE experienced will also be recorded. The severity of the AE will be graded according to the CTCAE v5.0: **Grade 1:** Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. **Grade 2:** Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)*. **Grade 3:** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**. **Grade 4:** Life-threatening consequences; urgent intervention indicated. **Grade 5:** Death related to AE. **ADL:** *Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

AE and SAE Recording
**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.
Assessment of Causality
The Investigator is obligated to assess the relationship between IP and each occurrence of each AE/SAE. • A “"reasonable possibility”" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. • The Investigator will use clinical judgment to determine the relationship. • Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to IP administration will be considered and investigated. • The Investigator will also consult the Investigator’s Brochure (IB) and/or Product Information, for marketed products, in his/her assessment. • For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality. • There may be situations in which an SAE has occurred and the Investigator has minimal information to include in the initial report to the Medical Monitor (or designee). However, **it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the Medical Monitor (or designee).** • The Investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment. • The causality assessment is one of the criteria used when determining regulatory reporting requirements. Causality Categories: • **Related** – The AE is known to occur with the IP, there is a reasonable possibility that the IP caused the AE, or there is a temporal relationship between the IP and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the IP and the AE. • **Not Related** – There is not a reasonable possibility that the administration of the IP caused the event, there is no temporal relationship between the IP and event onset, or an alternative etiology has been established.

AE and SAE Recording
Follow-up of AEs and SAEs
• The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Medical Monitor (or designee) to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. • If a subject dies during participation in the study or during a recognized follow-up period, the Investigator will provide the Medical Monitor (or designee) with a copy of any post-mortem findings including histopathology. • New or updated information will be recorded in the originally completed eCRF. • The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

10.3.6. Reporting of SAEs

Reporting of SAEs will be done using paper methods.

SAE Reporting to the Medical Monitor (or designee) via Paper CRF
• Facsimile or email transmission of the SAE paper CRF is the preferred method to transmit this information to the Medical Monitor (or designee). • In rare circumstances and in the absence of facsimile or email equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service. • Initial notification via telephone does not replace the need for the Investigator to complete and sign the SAE CRF pages within the designated reporting time frames. • Contacts for SAE reporting can be found in the relevant manual.

10.4. Appendix 4: Contraceptive Guidance and Collection of Pregnancy Information

Definitions:

Woman of Childbearing Potential (WOCBP)

Women who do not meet criteria for "females not of childbearing potential" (see below) and are post-menarche are considered to be "females of childbearing potential" in this study.

Women NOT of childbearing potential

1. Pre-menarchal
2. Surgically sterile (hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy, or bilateral tubal occlusion)
3. Postmenopausal (defined as cessation of regular menstrual periods for at least 12 months without an alternative medical cause in women over the age of 45).
    a. In women under the age of 45 who have amenorrhea >12 months, menopausal status may be confirmed by follicle stimulating hormone (FSH) level of ≥30.0 mIU/mL in women not using hormonal contraception or hormone replacement therapy. Note that FSH levels can be normal or in the postmenopausal range depending on degree of ovarian dysfunction in women under 45 years of age with <12 months amenorrhea. Consult with the Medical Monitor (or designee) regarding subjects whose menopausal status may be in transition or unclear.
4. For individuals with permanent infertility due to an alternative medical cause (e.g., Mullerian agenesis, androgen insensitivity), Investigator discretion should be applied to determining study entry.

Male subjects

Men will not be required to use contraception when sexually active with a woman who is not of childbearing potential.

Men who are sexually active with WOCBP need to confirm that they will use 2 forms of contraception as per protocol.

Men who are sexually active with a partner who is pregnant are required to use a condom.

Contraception Guidance:

Contraceptive use by men or women should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

Subjects should use highly effective, double contraception (both male and female partners) during the study and 30 days after the last dose of IP.

Double contraception is defined as a condom with one of any of the following:

- Oral contraceptive pills
- Depot or injectable birth control
- Intrauterine device (IUD)

- Contraceptive patch (e.g., Xulane®) or NuvaRing®
- Vasectomy

Subjects may not donate sperm or oocytes during the study and for 30 days or 5 half-lives (whichever is longer) after last dose of IP.

Rhythm methods are not considered as highly effective methods of birth control. Subject abstinence for the duration of the study and 30 days or 5 half-lives (whichever is longer) after last dose of IP is acceptable if it is the subject's regular practice.

Collection of Pregnancy Information

Male subjects with partners who become pregnant

- The Investigator will attempt to collect pregnancy information on any male subject's female partner who becomes pregnant while the male subject is in this study.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female subjects who become pregnant

- Female subjects who become pregnant should discontinue treatment and be withdrawn from the study.
- The Investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study. Information will be recorded on the appropriate form and submitted to the Sponsor within 24 hours of learning of a subject's pregnancy.
- The subject will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the subject and the neonate and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such. Any post-study pregnancy related SAE considered reasonably related to the IP by the Investigator will be reported to the Sponsor as described in Section 8.3.4. While the Investigator is not obligated to actively seek this information in former study subjects, he or she may learn of an SAE through spontaneous reporting.

10.5. Appendix 5: Pharmacogenomics

Use and Analysis of DNA

- Germ line variation may impact a subject's response to IP, susceptibility to, and severity and progression of disease. Variable response to IP may be due to genetic determinants that impact drug absorption, distribution, metabolism, and excretion; mechanism of action of the drug; disease etiology; and/or molecular subtype of the disease being treated. Therefore, where local regulations and IRB/IEC allow, a bio-sample may be collected for analysis from subjects.
- Samples may be analyzed for genetic variations in genes that significantly affect the PK of pegozafermin, the safety, and/or efficacy profile. Often times, a large variability in the plasma concentration–time profiles of any medicine can be linked to loss of function mutations in the drug metabolizing enzymes and/or transporters.
- If analyzed, the results of these analyses may be reported in a separate study summary.
- The Sponsor will store the DNA samples in a secure storage space with adequate measures to protect confidentiality.
- The samples will be retained while research on pegozafermin continues but no longer than 8 years or other period as per local requirements.

10.6. Appendix 6: Guidance to Address a Pandemic or Other Global Health Emergencies and Potential Impact on the Clinical Study

In the occurrence of a global health emergency affecting the conduct of the ongoing study, such as the coronavirus disease 2019 (COVID-19) pandemic, study conduct may be adjusted due to subjects being in self-isolation/quarantine, limited access to public places (including hospitals) due to the risk of spreading infections, and health care professionals being committed to critical tasks. Study conduct and/or assessments may also be impacted by secondary factors as a result of the pandemic, such as supply chain issues and logistical challenges. In instances where COVID-19 pandemic restrictions or logistical scheduling challenges may be of concern, adjustments to the study conduct may be necessary (e.g., the screening period may be extended beyond 12 weeks with Sponsor approval, study visit windows may also extended etc.) (FDA, August 2021).

Adjustments to the this protocol may be made as described below, in line with global regulatory authorities' guidance to ensure the safety of study subjects, maintain compliance with Good Clinical Practice (GCP), and minimize the risks to study integrity during the COVID-19 pandemic (Health Canada, 03 April 2020; MHRA, 22 April 2020; EMA, April 2020; FDA, March 2020). Other countries may issue their own guidance requiring country-specific recommendations to be followed.

Informed Consent

- If written consent by the study subject is not possible (e.g., because of physical isolation due to COVID-19 or other global health emergencies), consent could be given orally by the study subject and documented according to regulatory guidance.

- Study subjects and the person obtaining consent could sign and date separate Informed Consent Forms (ICF).
- In case written informed consent cannot be obtained at the clinical site, electronic informed consent can be obtained remotely. Alternatively, the consent form may be sent to the subject or the subject's legally authorized representative by facsimile or email, and the consent interview may then be conducted by telephone/teleconference when the subject or subject's legally authorized representative can read the consent form during the discussion; the subject or subject's legally authorized representative will be requested to sign and date a blank piece of paper with a written statement affirming that they agree to participate in the study and documented according to regulatory guidance.
- If re-consent is necessary for the implementation of **new urgent changes in study conduct** (mainly expected for reasons related to global health emergencies or important safety issues for other studies), alternative ways of obtaining consent may include contacting the study subject via phone or video-calls and obtaining verbal consent, to be documented in the study subjects' medical records, supplemented with e-mail confirmation.
- The informed consent procedure is to remain compliant with the study protocol as well as local regulatory requirements. All relevant records should be archived in the Investigator's site master file. A correctly signed and dated ICF should be obtained from the study subjects later, as soon as possible.

Study Visits and Procedures

- COVID-19 screening procedures that may be mandated by the health care system in which a clinical study is being conducted do not need to be reported as an amendment to the protocol even if done during clinical study visits. The Investigator in consultation with the Sponsor will decide if it is in the best interest of COVID19positive subjects to remain in the study.
- In the case of missed visits due to global health emergencies (or other pandemic-related reasons):
  - The site should make every effort to contact the study subject to confirm and document the reason for the missed visit and at minimum, evaluate Adverse Event (AEs)/Serious Adverse Events (SAEs), and concomitant medications to assess subject safety.
  - Upon resumption of visits, the subject will continue at the next planned visit relative to the last visit performed prior to study interruption.
- Changes in study visit schedules, missed visits, or subject discontinuations may lead to missing information (e.g., for protocol-specified procedures). Specific information will be captured in the case report form that explains the basis of the missing data, including the relationship to COVID19 for missing protocol-specified information (e.g., from missed study visits or study discontinuations due to COVID-19).

- To maintain the integrity of the study, alternative methods of collecting study procedures may be considered where possible:
  - In cases where global health emergencies-related circumstances preclude a visit to the investigative site, remote visits (e.g., by telemedicine or phone contact) will be allowed for relevant study procedures.
  - In certain situations, with Sponsor approval, and according to site business continuity plans, remote/home visits may be used, e.g., to collect laboratory samples and assessments as required by the protocol.
  - Study assessments will be conducted in a remote manner if they can be done without affecting the well-being of the subject during the study.
  - Remote study assessments can be completed via online technology. The subject may interact with study personnel using online communication tools that incorporate telemedicine.
  - In cases where a subject is continuing to receive IP but cannot obtain laboratory tests at the investigative site, local laboratory tests may be obtained at a certified laboratory. The site should inform the Sponsor about such cases. Local analysis can be used for safety decisions. In addition, local labs can be used for trial endpoints if samples drawn at the local laboratory cannot be shipped to the central laboratory.
  - Urine pregnancy tests can be performed if serum pregnancy tests cannot be performed.

Investigational Product and/or Study Interruption Due to Global Pandemic

- Interruption in IP administration and other study procedures, including MRI-PDFF and liver biopsy (collectively "study interruption") will be allowed for up to 4 weeks (+4 days). Any interruption lasting ≥2 weeks will be considered a study interruption.
- For subjects who have a study interruption, prior to resuming dosing, the Investigator will assess whether the subject is clinically stable to continue participation in the study. This assessment will include limited physical examination, vital signs, pregnancy test (local) in WOCBP, and laboratory tests (hematology and clinical biochemistry) to be done locally. If clinical laboratory tests are not part of the protocol-defined procedures for the first dosing visit after study interruption, hematology and clinical biochemistry panels will be obtained prior to dosing and sent to the central laboratory. Results from both the local and central labs should be documented in electronic case report form. Upon resumption of dosing, the subject will continue at the next planned visit relative to the last visit performed prior to study interruption and will be administered all remaining planned doses.
- End of Treatment may be considered for the following scenarios:
  - For subjects who have reached the Week 24 (Main study) or Week 48 (Extension study), an End of Treatment visit may be considered instead of the study interruption. The Investigator should discuss with the Sponsor about the

feasibility of these two options based on the potential impact from COVID-19 and/or the control measures in place in their location.

  - Study interruptions longer than 4 weeks will lead to IP discontinuation and the subject's withdrawal from the study.

- Efforts should be made to obtain End of Treatment assessments in subjects who are not willing to return to the study after study interruption, who are deemed not clinically stable to continue participation by the Investigator, or who are lost to the study due to disruptions because of COVID-19 pandemic. The End of Treatment visit should be scheduled as soon as possible in these scenarios.
- For dose interruptions lasting ≥2 weeks, contact will be established with the study subject remotely (e.g., by phone) to obtain information about AEs, concomitant medications, or any other update related to the subjects' safety.
- Study interruption will be allowed as long as COVID-19 pandemic-related circumstances are ongoing and will not be allowed when these circumstances are no longer applicable, as determined by the Sponsor.
- For newly randomized subjects who have not yet received their first dose at a site that can foresee near-term disruption by COVID-19 pandemic, the Investigator should discuss with the Sponsor whether the initiation of dosing should be postponed. In this situation, study interruption should not apply, and the subject will initiate and finish all planned doses when the Investigator deems it is safe to start dosing.

Supply of Investigational Product

- IP supply will be provided for home administration as planned in the protocol.
- Sponsor approved IP supply options will be specified in the Pharmacy Manual. Direct-to-patient shipment may be available, where possible. Direct-to-patient IP shipment will require consent to provide name and address to courier.

Monitoring and Audits

- Certain Sponsor oversight responsibilities, such as monitoring and quality assurance activities, may need to be re-assessed and temporary, alternative proportionate mechanisms of oversight may be required. On-site audits will be avoided or postponed, and if permitted under local regulations, social distancing restrictions should apply.
- Canceling or postponing on-site monitoring visits and extending the period between monitoring visits will be allowed.
- To the extent on-site monitoring remains feasible, it should take into account national, local, and/or organizational social distancing restrictions.
- Centralized monitoring can be considered for data acquired by electronic data capture systems (e.g., electronic case report forms [eCRFs], central laboratory or electrocardiogram [ECG] data, electronic patient reported outcomes) that are in place or could be put in place, providing additional monitoring capabilities that can

supplement and temporarily replace on-site monitoring through remote evaluation of ongoing and/or cumulative data collected from study sites, in a timely manner.

- Off-site monitoring can be conducted and will include phone calls, video visits, e-mail, or other online tools to discuss the study with the Investigator and site staff. Remote monitoring should be focused on review of critical study site documentation and source data. These activities could be used to get information on the clinical study progress, to exchange information on the resolution of problems, review of procedures, study subject status, as well as to facilitate remote site selection and Investigator training for critical study procedures.

Risk Mitigation

The Sponsor will continually assess whether the limitations imposed by the COVID-19 public health emergency on protocol implementation pose new safety risks to study subjects, and whether it is feasible to mitigate these risks by amending study processes and/or procedures.

10.7. Appendix 7: Prescreening Criteria for Identifying Potential Subjects with NASH and Stage 2 or 3 Fibrosis

To minimize unnecessary biopsies and help sites identify subjects at high risk for NASH with Stage 2 or 3 fibrosis (per NASH CRN system), the Sponsor recommends that screening liver biopsy decisions be based on the following prescreening criteria.

These guidelines are not inclusionary protocol requirements but are recommendations that Investigators can use when considering whether a potential subject should be screened for the study. All efforts should be made to ensure that noninvasive procedures are performed prior to the liver biopsy.

Consult with the Medical Monitor (or designee) if clarification is needed on whether a potential subject should be considered for the study.

1. **Increased metabolic risk**: potential subjects with at least 2 of the criteria as part of their medical history are at increased metabolic risk:
    a. Central obesity: Waist circumference of >102.0 cm for males, >88.0 cm for females, or body mass index (BMI) >25.0 kg/m^2. BMI should not exceed 50.0 kg/m^2.
    b. Type 2 diabetes mellitus (T2DM; as determined by medical history ≥3 months before Screening); or fasting glucose ≥100 mg/dL
    c. Increased fasting triglycerides (≥150 mg/dL) or on treatment for hypertriglyceridemia
    d. Reduced fasting HDL-c (<40 mg/dL for males and <50 mg/dL for females)
    e. Hypertension or on treatment for hypertension; or ≥130 mmHg systolic blood pressure or ≥85 mmHg diastolic blood pressure

2. **Increased risk for Stage 2 or 3 fibrosis**: potential subjects meeting one of the following criteria have a high likelihood of having Stage 2 or 3 fibrosis:
    a. Fibroscan VCTE score ≥7.0 kPa.
    b. A historical liver biopsy that was performed more than 6 months but less than 2 years before Screening that indicated Stage 1, 2 or 3 fibrosis. While this historical biopsy would not be eligible for the study, there is high likelihood that a new screening biopsy would indicate Stage 2 or 3 fibrosis.
    c. AST value >30 U/L

3. **Increased risk for steatosis**: potential subjects meeting one of the following criteria have a high likelihood of having steatosis:
    f. MRI-PDFF ≥5.0%
    a. Where available, sites may perform Fibroscan controlled attenuation parameter (CAP) measurements to estimate the risk for steatosis, as CAP is correlated with fat content. Potential subjects with CAP values ≥250 dB/m are likely to be at high risk for steatosis. To ensure accuracy, Fibroscan assessments should always be performed with a subject-appropriate probe and according to manufacturer's instructions.

10.8. Appendix 8: Child-Turcotte-Pugh Classification for Severity of Liver Disease

Points:	1	2	3
Encephalopathy	None	Grade 1 or Grade 2	Grade 3 or Grade 4
Ascites	Absent	Slight	Moderate
Bilirubin (mg/dL)	<2	2 to 3	> 3
Albumin (g/dL)	> 3.5	2.8 to 3.5	< 2.8
Prothrombin Time (INR)	<1.7	1.7 to 2.3	>2.3

Note: CTP Score is obtained by adding the score for each parameter. CTP Score interpretation is as follows:
- 5 to 6 points: Child class A
- 7 to 9 points: Child class B
- 10 to 15 points: Child class C

References:

1. Pugh RN, Murray-Lyon IM, Dawson JL, et al. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg. 1973; 60:646
2. Child CG, Turcotte JG. The Liver and Portal Hypertension. Philadelphia, WB Saunders Co. 1964.
3. Trey C, Burns DG, Saunders SJ. Treatment of hepatic coma by exchange blood transfusion. NEJM. 1966; 274:473

10.9. Appendix 9: Glossary

Abbreviation Term	Description
ADA	Antidrug antibodies
ADL	Activities of Daily Living
AE	Adverse event
AFP	Alpha feto-protein
AI	Artificial intelligence
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANCOVA	Analysis of variance
AST	Aspartate transaminase
AUC	Area under the curve
BMD	Bone mineral density
BMI	Body mass index
CAP	Controlled Attenuation Parameter
CFR	Code of Federal Regulations
CI	Confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CKD-EPI	Chronic kidney disease-epidemiology
CLDQ-NAFLD-NASH	Chronic Liver Disease Questionnaire - NAFLD-NASH
CMH	Cochran-Mantel-Haenszel
COVID-19	Coronavirus disease 2019
CRN	Clinical Research Network
cT1	Iron-corrected T1 mapping
CTCAE	Common terminology criteria for adverse events
CTP	Child-Turcotte-Pugh
CTX	Carboxy-terminal collagen crosslinks
DILI	Drug-induced liver injury
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
DXA	Dual X-ray absorptiometry
DST	Dexamethasone suppression test
ECG	Electrocardiogram
eCRF	Electronic case report form
ELF	Enhanced liver fibrosis
EOS	End of study
EQ-5D-5L	Europe Quality of Life Group 5-dimension 5-level questionnaire

Abbreviation Term	Description
ET	Early Termination
FAS	Full Analysis Set
FDA	Food and Drug Administration
FGF21	Fibroblast growth factor 21
FGFR	Fibroblast growth factor receptor
FIB-4	Fibrosis-4
FSH	Follicle stimulating hormone
FU	Follow up
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
GI	Gastrointestinal
HA	Hyaluronic acid
HbA1c	Glycated hemoglobin
HCV	Hepatitis C virus
HDL	High density lipoprotein
HDL-c	High density lipoprotein cholesterol
HIV	Human Immunodeficiency Virus
HOMA-IR	Homeostatic model assessment for insulin resistance
IB	Investigator's Brochure
ICE	Intercurrent event
ICF	Informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IGF-1	Insulin-like growth factor-1
INR	International normalized ratio
IP	Investigational product
IRB	Institutional Review Board
IRT	Interactive Response Technology
ISR	Injection site reaction
kPa	Kilopascal
LDL-c	Low density lipoprotein cholesterol
LS	Least square
MCH	Mean corpuscular hemoglobin
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed model repeated measures
MRI	Magnetic resonance imaging

Abbreviation Term	Description
n	Number
NAb	Neutralizing antibodies
NAFLD	Nonalcoholic fatty liver disease
NAS	NAFLD Activity Score
NASH	Nonalcoholic steatohepatitis
NFS	NAFLD fibrosis score
NOAEL	No observed adverse effect level
NSC	Night-time salivary cortisol
P1NP	N-terminal propeptide of type 1 collagen
P3NP	Amino-terminal type 3 procollagen peptide
PD	Pharmacodynamic
PDFF	Proton density fat fraction
PEG	Polyethylene glycol
PGx	Pharmacogenomics
PK	Pharmacokinetic
PLT	Platelet
PNASH	Phenotypic NASH, defined as at least one of the following: 1) obesity with T2DM. 2) Obesity with evidence of liver injury (either increased alanine aminotransferase [ALT] and/or vibration-controlled transient elastography [VCTE] score ≥7 KPa)
PRO	Patient reported outcome
Pro-C3	N-terminal propeptide of type 3 collagen
Q2W	Every 2 weeks
QRS	Complex in ECG representing ventricular depolarization
QTcF	Fredericia corrected QT interval in ECG
QW	Weekly
RNA	Ribonucleic acid
SAD	Single ascending doses
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
SD	Standard deviation
SE	Standard error
SoA	Schedule of activities
SUSAR	Suspected unexpected serious adverse event
T2DM	Type 2 diabetes mellitus
TB	Total bilirubin

Abbreviation Term	Description
TEAE	Treatment-emergent adverse event
t_{max}	Time to achieve maximal observed serum concentrations (C_{max})
TSH	Thyroid stimulating hormone
UFC	Urinary free cortisol
ULN	Upper limit of normal
US	United States
VAS	Visual analog scale
VCTE	Vibration-controlled transient elastography
WOCBP	Woman of childbearing potential
WPAI-NASH	Work Productivity and Activity Impairment Questionnaire for NASH

10.10. Appendix 10: Protocol Amendment History

The Protocol Amendment Summary of Changes for the current amendment is located directly before the Table of Contents. The Summary of Changes for prior protocol amendments are presented in this appendix.

Amendment 2: Version 3.0 (11 March 2022)

Section Number and Heading	Description of Change	Brief Rationale
1.1 Synopsis and 1.2 Study Schema	All applicable changes made in the corresponding sections in the protocol were included in the synopsis and study schema.	See below for the rationale for specific changes.
3.1 Main Study Objectives and Endpoints 9.1 Statistical Hypotheses	• Primary endpoint has been modified by the addition of ≥ 2-point reduction in NAS score: (i.e., *Proportion of subjects with NASH resolution without worsening of fibrosis and ≥ 2-point improvement in NAS score at Week 24 compared to baseline* **OR** *Proportion of subjects achieving improvement of fibrosis ≥1 stage without worsening of NASH and ≥ 2-point improvement in NAS score at Week 24 compared to baseline*) • Additional Key secondary endpoint: (i.e., *Proportion of subjects with ≥ 2-point improvement in NAS score AND are MRI-PDFF responders AND ALT responders at Week 24 compared to baseline*) and additional exploratory endpoints that include histology and/or MRI-PDFF responders, and/or ALT responders to the composite	• The modified Primary endpoint allows for stabilization of placebo response to improve ability to determine treatment effect with pegozafermin • ≥ 2-point reduction in NAS components reflect decrease in disease severity thus are clinically meaningful • Responses in MRI-PDFF (i.e., reduction by ≥30%) and ALT (i.e., reduction by ≥17 U/L or ≥30%) are clinically meaningful and have been previously associated with positive histological outcomes
4 Study Design 9.2 Sample Size Determination	• Overall sample size reduction (from 216 to 184) and redistribution among arms	• Preliminary data from Part 2 of the Phase 1b/2a study suggest that 30 mg QW pegozafermin dose may be highly active, thus subject numbers have been increased in both the 30 mg QW active and placebo QW arms • Sample sizes at the 15 mg QW and 44 mg Q2W pegozafermin have been reduced to accommodate increases to the 30 mg QW arms, yet remain at sufficient levels to generate meaningful data at these lower doses
5.2 Exclusion Criteria	• Added clarification to the language for Exclusion Criteria # 1, #16, # 17, and # 21. • Added alkaline phosphatase >2-fold higher than ULN and triglycerides > 1000 mg/dL as exclusionary laboratory abnormalities in EC #18.	• Clarification • Added limits on allowable ULN for alkaline phosphatase and triglycerides to exclude subjects at risk for other possibly confounding comorbidities and safety events

Section Number and Heading	Description of Change	Brief Rationale
8.2.5.3 Cortisol Assessments (new)	• Added further details on the instructions for NSC collection and assessment.	• Clarification
Overall	• Editorial and formatting changes, including updates to references. • The term BIO89-100 was replaced with the USAN 'pegozafermin' throughout the protocol, as applicable. • Change in Sponsor signatory.	• To clarify and correct any errors or inconsistencies across sections. • Update • Personnel update

Amendment 1: Version 2.0 (01 December 2021)

Section Number and Heading	Description of Change	Brief Rationale
1.1 Synopsis and 1.2 Study Schema	All applicable changes made in the corresponding sections in the protocol were included in the synopsis and study schema.	See below for the rationale for specific changes.
1.3 Schedule of Activities (SoA)	• Added blood sample collection for exploratory biomarkers at Day 1, Week 24, Week 48/ ET visits. • Removed the requirement to perform assessments predose for several assessments at specific visits as shown in the SoA. • Revised Week 26 visit to be either in clinic or remote visit, rather than remote only. • Clarified the language on process, timing, requirements, or description for the following assessments: NSC, urine drug screen, serology, biochemistry, FSH, vital signs, liver biopsy, MRI-PDFF, and immunogenicity. • Specified that the screening window may be extended with Sponsor's approval and certain assessments (MRI-PDFF, DXA) if needed to be performed out of specified window, should be done with the Medical Monitor (or designee's) approval. • Specified that strenuous exercises should be avoided for at least 48 hours prior to study visits.	• Inadvertently omitted from the SoA. • To provide flexibility in the sequence of assessments. • To provide visit flexibility to sites and subjects. • To clarify and provide further guidance on operational and logistics aspects of assessments. • To provide flexibility in scheduling for the various assessments at screening and other visits. • To avoid potential confounding effect on study assessments.
2.3.1 Risk Assessment	Removed the detailed information on risk assessment including Table 2 and cross-referenced the IB for this information.	For consistency and reference to most current information on BIO89-100.
4. Study Design	• Specified that the Extension study will be single-blind, i.e., the investigator (including site staff) and subject will be blinded but the Sponsor will not be blinded. • Added that the screening period may be extended with Sponsor's approval.	• For continued planning of the development program for BIO89100 and Sponsor interactions with the Agency. • To provide flexibility.
5.1 Inclusion Criteria	IC 3: Simplified criterion for eligibility with biopsy and listed the specific parameters (revised 3.b.1 and 3.b.2) to consider prior to biopsy as guidance for prescreening criteria in Appendix 7.	To clarify criteria and loosen the otherwise stringent criteria, to facilitate enrolment.

Section Number and Heading	Description of Change	Brief Rationale
	IC 4: Removed as a protocol IC and listed as a prescreening criterion (Appendix 7) for identifying subjects with increased risk for steatosis, with revised thresholds for MRI-PDFF and CAP scores.	
5.2 Exclusion Criteria	EC 1: Provided additional guidance on eligibility criterion for subjects with prior HCV infection. EC 2: Added planned liver transplantation to EC. EC 3: Revised the parameters to consider as evidence of cirrhosis. EC 5: Specified that a positive COVID-19 test, or COVID-19 diagnosis is not exclusionary. EC 8: Revised timeframe for the definition of newly diagnosed hypertension from '< 3' to '< 2' months since screening and removed the provision for allowing newly diagnosed subjects who had already initiated treatment or subjects on more than 2 antihypertensives. EC 10: Clarified language regarding stable antidiabetic regimen for T2DM. EC 12: Clarified language regarding weight loss plan to include initiation of weight loss program, training for marathon, and weight loss medication during the study. EC 14: Provided additional details on other bone disorders. EC 15: Clarified language around history of malignancy and the timeframe for exclusion. EC 16: Further clarification provided on definition of significant alcohol consumption. EC 17: Clarified criterion on substance use disorder and the version of DSM used to define disorder. EC 18: Revised language to allow for more than one repeat test, removed alkaline phosphatase test from the list of exclusionary lab abnormalities, and specified the interval between 2nd and 3rd ALT and AST screening assessments. EC 22 (old): Removed the exclusionary criterion as it is difficult to quantify cannabis use. EC 24 (new): specified the dose for Vitamin E and clarified timeframe for stable dose.	The changes specified for the various EC were made to either clarify criteria, provide additional information, or to loosen the otherwise stringent criteria, in order to facilitate enrolment.
5.3 Lifestyle Considerations	• Clarified the recommended guidelines for alcohol intake. • Specified that strenuous exercises should be avoided for at least 48 hours prior to study visits. • Added that activities such as starting a new weight loss program, training for a marathon, or taking a concomitant weight loss medication are exclusionary (EC # 12).	For clarification and additional information.
5.4 Screen Failures and Rescreening	Included information on when retests are allowed during the screening period and added criteria and details for when a subject can be rescreened.	For clarification and additional information.

Section Number and Heading	Description of Change	Brief Rationale
6. Investigational Product	• Clarified that a subject's caregiver may administer IP following training.	For clarification and additional information.
6.4 Measures to Minimize Bias: Randomization and Blinding	• Specified that the proportion of subjects enrolled with MRIPDFF <8% may be limited at the Sponsor's discretion. • Added that subjects who are rescreened will retain their original ID number; a new ID number will not be assigned. • Specified that the Extension study will be single blind and removed reference to a study-data integrity plan that was to be created for selected unblinding. • Clarified that the DMC will have access to unblinded safety data (not emerging efficacy results) for review purposes.	For clarification and additional information.
6.7 Concomitant Therapy	Added that short-term use of sliding scale insulin therapy during a medical procedure or hospitalization (<5 days) for subjects with T2DM may be performed as needed.	For clarification and additional information.
6.7.1 Prohibited Medications/ Therapies	• Clarified the prohibited and acceptable criteria for antidiabetic medications. • Specified the dose for Vitamin E that is prohibited. • Added that weight loss medication should not be initiated during the study.	For clarification.
7.1 Discontinuation of IP and Subject Withdrawal	Added that for any subject experiencing a Grade 3 TEAE that is considered related to IP, the Investigator should discuss treatment discontinuation with the Medical Monitor (or designee).	For additional guidance on handling of discontinuations.
8.1.1 Liver Biopsy and Scoring	• Provided further guidance on when to perform biopsy and if not performed within the given window. • Included information on the NASH CRN scoring of histological features with reference.	For clarification and additional information.
8.1.2 MRI-PDFF	• Specified that 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. This also applies to cT1, pancreatic fat, liver-volume, and spleen-volume assessments. • Specified that the proportion of subjects enrolled with MRI-PDFF <8% may be limited at the Sponsor's discretion.	To provide flexibility and facilitate enrolment.
8.1.3 Transient Elastography kPa and CAP scores	Specified that a historical Fibroscan assessment performed within the last 3 months prior to screening may be acceptable.	To provide flexibility and facilitate enrolment.
9.4.2 Safety Analyses	Specified the TEAE reporting period for subjects who withdraw consent from the study.	For clarification.
9.6 Primary Analysis	Removed the reference to a study data integrity plan for unblinding required for unblinding select sponsor personnel.	Sponsor will be unblinded for the Extension study.

Section Number and Heading	Description of Change	Brief Rationale
9.7 DMC	Revised to specify that DMC will review unblinded safety data and not emerging efficacy results.	Correction.
10.2 Appendix 2	Clarified the specifications for FSH, NSC, urine drug screen, and serology assessments.	For clarification and further guidance on assessments.
10.8 Appendix 7 (new)	Included prescreening criteria as guidelines and recommendations for identifying potential subjects for the study. These are adapted (with revisions) from IC 3.b.1., 3.b.2, and 4 in the original protocol (v1.0).	To facilitate recruitment and enrolment.
Overall	Editorial and formatting changes, including updates to references.	To clarify and correct any errors or inconsistencies across sections.

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