CLINICAL STUDY PROTOCOL:

A PHASE 2B, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY EVALUATING THE SAFETY AND EFFICACY OF EFRUXIFERMIN IN SUBJECTS WITH COMPENSATED CIRRHOSIS DUE TO NONALCOHOLIC STEATOHEPATITIS (NASH)

Protocol Number:	AK-US-001-0103
IND Number:	140307
Clinical Trials.gov Identifier:	NCT05039450
EudraCT Number:	Not applicable
CRO Medical Monitor:	[redacted]
Sponsor:	Akero Therapeutics, Inc. 601 Gateway Blvd., Suite 350 South San Francisco, CA 94080
Version and Date:	Original, 20 May 2021 Amendment 1, 15 October 2021 Amendment 2, 21 December 2021 Amendment 3, 14 February 2022 Amendment 4, 17 March 2022 Amendment 5, 11 August 2022 Amendment 6, 15 February 2023

CONFIDENTIALITY STATEMENT

Information contained in this protocol is proprietary and confidential in nature and may not be used, divulged, published, or otherwise disclosed to others except to the extent necessary to obtain approval of the Institutional Review Board or as required by law. Persons to whom this information is disclosed should be informed that this information is confidential and may not be further disclosed without the express permission of Akero Therapeutics, Inc.

STUDY ACKNOWLEDGEMENT SIGNATURE PAGE

A Phase 2b, Randomized, Double-Blind, Placebo-Controlled Study Evaluating the Safety and Efficacy of Efruxifermin in Subjects with Compensated Cirrhosis Due to Nonalcoholic Steatohepatitis (NASH)

AK-US-001-0103, Amendment 6, 15 February 2023

This clinical study protocol was subject to critical review and has been approved by the Sponsor.

[redacted] Feb 23rd 2023

Date

INVESTIGATOR STATEMENT

I have read the protocol, including all appendices, and I agree that it contains all necessary details for me and my staff to conduct this study as described. I will conduct this study as outlined herein and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and access to all information provided by Akero Therapeutics, Inc. I will discuss this material with them to ensure that they are fully informed about the drug(s) and the study.

Principal Investigator Name (Printed) Signature

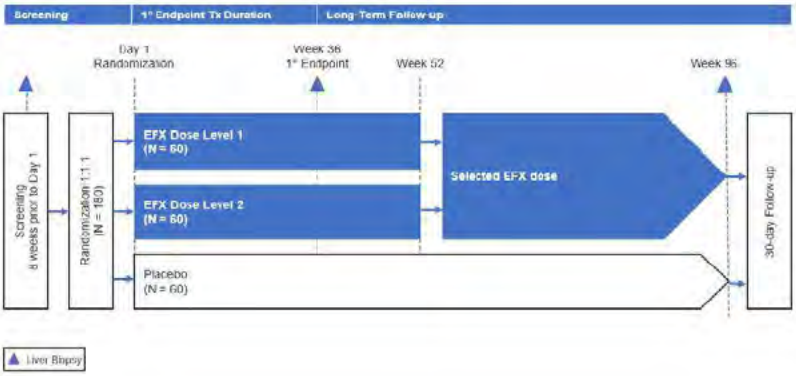
Date

SYNOPSIS

Name of Sponsor Company:	Akero Therapeutics, Inc.
Name of Test Product and Dosage:	Efruxifermin (EFX) 50 mg and 28 mg
Title of Study:	A Phase 2b, Randomized, Double-Blind, Placebo-Controlled Study Evaluating the Safety and Efficacy of Efruxifermin in Subjects with Compensated Cirrhosis Due to Nonalcoholic Steatohepatitis (NASH)
Phase of Development:	Phase 2b
Investigator and Study Centers:	Approximately 60 centers in North America
Study Period:	**Main Study** Participation will include: • Screening: 8 weeks • Primary Endpoint Treatment Duration: 36 weeks • Long-Term Follow-up: up to 60 weeks after the Primary Endpoint Treatment Duration (i.e., up to 96 weeks of total treatment duration) • 30-day Follow-up: approximately 30 days after the last dose of study drug **Cohort D** Participation will include: • Screening: 8 weeks • Treatment Duration: 12 weeks • 30-day Follow-up: approximately 30 days after the last dose of study drug
Objectives:	**Main Study** The primary objective of this study is: • To evaluate the effect of EFX compared to placebo on fibrosis regression in subjects with compensated cirrhosis due to NASH at Week 36 The secondary objectives of this study are:

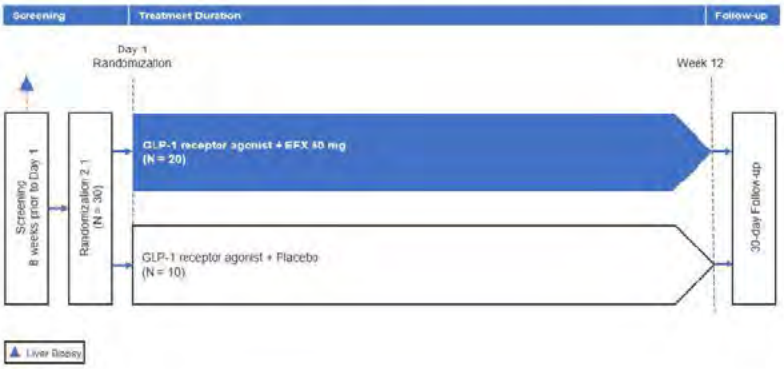
	• To evaluate the effect of EFX compared to placebo on achieving NASH resolution in subjects with compensated cirrhosis due to NASH at Week 36 and Week 96 • To evaluate the effect of EFX compared to placebo on achieving NASH resolution AND improvement in fibrosis at Week 36 and Week 96 • To evaluate the effect of EFX compared to placebo on fibrosis regression in subjects with compensated cirrhosis due to NASH at Week 96 • To evaluate the effect of EFX compared to placebo on fibrosis regression in subjects with compensated cirrhosis due to NASH at Week 36 and Week 96 • To evaluate the effect of EFX compared to placebo on non-invasive markers of fibrosis • To evaluate the effect of EFX compared to placebo on lipoproteins • To evaluate the effect of EFX compared to placebo on markers of insulin sensitivity and glycemic control • To evaluate the effect of EFX compared to placebo on weight change • To assess the immunogenicity of EFX • To assess the safety and tolerability of EFX **Cohort D** The primary objective of this cohort is: • To evaluate the safety and tolerability of EFX compared to placebo when added to an existing glucagon-like-peptide-1 (GLP-1) receptor agonist in subjects with type 2 diabetes (T2D) and liver fibrosis due to NASH The secondary objectives of this study are: • To evaluate the effect of EFX compared to placebo when added to an existing GLP-1 receptor agonist on liver fat content • To evaluate the effect of EFX compared to placebo when added to an existing GLP-1 receptor agonist on markers of liver injury and function, and hemostasis

	• To evaluate the effect of EFX compared to placebo when added to an existing GLP-1 receptor agonist on non-invasive markers of fibrosis • To evaluate the effect of EFX compared to placebo when added to an existing GLP-1 receptor agonist on lipoproteins • To evaluate the effect of EFX compared to placebo when added to an existing GLP-1 receptor agonist on markers of insulin sensitivity and glycemic control • To evaluate the effect of EFX compared to placebo when added to an existing GLP-1 receptor agonist on weight change • To assess the immunogenicity of EFX For a complete list of study objectives, refer to Section 2.
Study Design:	**Main Study** This is a Phase 2b, randomized, double-blind, placebo-controlled study evaluating the safety and efficacy of EFX in subjects with compensated cirrhosis due to NASH. Subjects will have fibrosis stage 4 without any history of decompensation and will be dosed once weekly (QW) with EFX 50 mg, EFX 28 mg, or placebo for up to 96 weeks. After all subjects have completed the Primary Endpoint Treatment Duration (i.e., completed 36 weeks of treatment or permanently discontinued the study prior to the Week 36 visit), the primary endpoint analysis will be performed to select a single dose of EFX (50 mg or 28 mg) for further evaluation. The Sponsor will seek alignment from the applicable regulatory authorities on the dose selected prior to clinical use. If a subject refuses to complete the Week 36 liver biopsy, the subject will be discontinued from the study at Week 36. Only a 30-day Follow-up visit is required following discontinuation of study drug. Subjects receiving EFX 50 mg or EFX 28 mg may be eligible to receive the selected dose of EFX. Roll over to the selected dose of EFX will occur only after all active subjects have completed 52 weeks of treatment and dose selection is confirmed. Subjects assigned to the placebo group will continue to receive placebo but may be offered open-label EFX in a separate roll over study after completing 96 weeks of treatment.



Cohort D

Cohort D is a randomized, double-blind, placebo-controlled cohort evaluating the safety and tolerability of EFX compared to placebo when added to an existing GLP-1 receptor agonist in subjects with T2D and liver fibrosis (stages 1—3) due to NASH. Subjects will be dosed QW with EFX 50 mg or placebo for up to 12 weeks.



Randomization:

Main Study

On Day 1, eligible subjects will be randomized in a blinded fashion in a 1:1:1 ratio to 1 of 3 treatment groups. Randomization will be stratified by T2D status (Yes vs No) and diagnosis of cryptogenic cirrhosis presumed secondary to NASH (Yes vs No) at baseline.

Treatment Group	Study Drug Assignment
EFX 50 mg (N = 60)	EFX 50 mg, subcutaneous injection, once weekly
EFX 28 mg (N = 60)	EFX 28 mg, subcutaneous injection, once weekly
Placebo (N = 60)	Placebo, subcutaneous injection, once weekly

<table>
<tr><td></td><td>EFX = Efruxifermin
Cohort D
On Day 1, eligible subjects will be randomized in a blinded fashion in a 2:1 ratio to 1 of 2 treatment groups as shown below. Randomization will be stratified by liver fibrosis stage (1 vs 2-3) at baseline.
<table>
<tr><th>Treatment Group</th><th>Study Drug Assignment</th></tr>
<tr><td>EFX 50 mg
(N = 20)</td><td>EFX 50 mg, subcutaneous injection, once weekly</td></tr>
<tr><td>Placebo
(N = 10)</td><td>Placebo, subcutaneous injection, once weekly</td></tr>
</table>
EFX = Efruxifermin</td></tr>
<tr><td>Number of Subjects Planned:</td><td>Main Study
Approximately [redacted] subjects
Cohort D
Approximately [redacted] subjects</td></tr>
<tr><td>Target Population:</td><td>Main Study
Males and non-pregnant, non-lactating females between 18–75 years of age with biopsy-proven compensated cirrhosis (fibrosis stage 4) due to NASH, and no evidence or history of hepatic decompensation. Subjects with cryptogenic cirrhosis presumed secondary to NASH will be limited to approximately 20% of the total study population.
Cohort D
Males and non-pregnant, non-lactating females between 18–75 years of age with biopsy-proven liver fibrosis (stages 1—3) due to NASH and T2D requiring treatment with a GLP-1 receptor agonist. The dose of the GLP-1 receptor agonist must be stable for at least 90 days prior to screening.</td></tr>
<tr><td>Diagnosis and Main Criteria for Inclusion:</td><td>To minimize risk and overall subject burden, it is recommended that invasive procedures, such as collection of the screening liver biopsy specimen, are performed after confirming that all other study entry criteria have been met.
If there is reasonable belief that an exclusionary result was obtained in error or is transient, a single repeat may be permitted with prior written approval from the Medical Monitor; with the exception of vital signs and ECG, which may be repeated one time</td></tr>
</table>

	at the Investigator's discretion. In addition, subjects who are ineligible (i.e., screen failures) may be rescreened once with prior written approval from the Medical Monitor. **Inclusion Criteria, Main Study** 1. Males and non-pregnant, non-lactating females between 18 and 75 years of age, inclusive, on the day of signing informed consent 2. Previous history or presence of T2D or 2 out of 4 components of metabolic syndrome (obesity, dyslipidemia, elevated blood pressure, elevated fasting glucose) 3. Subjects who do not have a historical liver biopsy specimen, which is defined as a liver biopsy specimen collected ≤ 365 days prior to screening with prior confirmation of fibrosis stage 4 that is available for submission to a central reader for eligibility assessment, must meet either inclusion criterion 3a **OR** 3b prior to collection of a liver biopsy specimen during the Screening visit: a. Liver stiffness measurement (LSM) [redacted] **Note**: All subjects must complete a FibroScan® examination during the screening period, unless historical values for FibroScan® performed within 90 days prior to the Screening visit are available b. Enhanced Liver Fibrosis (ELF) score [redacted] **Note:** Subjects who do not have a historical liver biopsy specimen require a screening ELF score. Historical results for an ELF test performed within 90 days prior to the Screening visit may be accepted as the screening value 4. Central laboratory tests at screening that meet all of the following criteria: a. Albumin ≥ 3.5 g/dL b. Estimated glomerular filtration rate (eGFR) ≥ 45 mL/min, as calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation c. Hemoglobin A1c (HbA1c) ≤ 9.5% d. International Normalized Ratio (INR) ≤ 1.3, unless due to therapeutic anticoagulation e. Direct bilirubin ≤ 0.3 mg/dL

	f. Total bilirubin ≤ 1.3 mg/dL, unless due to an alternate etiology such as Gilbert's syndrome or hemolytic anemia g. Creatine kinase (CK) < 3 × upper limit of normal (ULN) h. Platelet count ≥ 100,000/μL i. Triglyceride (TG) level ≤ 500 mg/dL j. Alanine aminotransferase (ALT) ≤ 5 × ULN k. Aspartate aminotransferase (AST) ≤ 5 × ULN l. Alkaline phosphatase (ALP) < 1.5 × ULN 5. Documented historical stability of ALT and AST levels, as evidenced by no significant worsening of ALT and AST values at screening relative to historical values obtained 28 days to 180 days prior to the Screening visit and based on the following parameters: a. If the historical and screening ALT and AST values are both ≤ 1.5 × ULN, there is no limit to the difference between the values b. If at least 1 of the historical or screening ALT or AST values is > 1.5 × ULN and shows worsening at screening, the percent increase must be ≤ 50% **Note**: Subjects without historical ALT and AST evaluations must have ALT and AST repeated during the screening period (Pre-baseline visit) at minimum 28 days between blood draws to confirm criterion 5a or 5b above 6. Biopsy-proven compensated cirrhosis (fibrosis stage 4) due to definitive NASH or cryptogenic cirrhosis presumed secondary to NASH as defined in Appendix G. Must have had a liver biopsy specimen collected during screening or ≤ 365 days prior to screening with confirmation of fibrosis stage 4 by a central pathologist. Subjects with cryptogenic cirrhosis presumed secondary to NASH will be limited to approximately 20% of the total study population 7. Subjects on Vitamin E ≥ 800 IU/day, antidiabetic, weight loss, or lipid-modifying medication(s) must be on a stable dose (defined as no significant change in prescription efficacy, initiation of medication, or medication discontinuation) as outlined in Table 6. A switch from one drug to another in the same class should be discussed with the Medical Monitor to confirm subject eligibility

	8. Willing and able to give written informed consent prior to any study specific procedures being performed 9. Female subjects of childbearing potential (see definition in Appendix D) must have a negative serum pregnancy test at screening and a negative urine pregnancy test at baseline/Day 1 10. Male and female subjects of childbearing potential who engage in heterosexual intercourse must agree to use protocol specified method(s) of contraception as described in Appendix D **Inclusion Criteria, Cohort D** 1. Males and non-pregnant, non-lactating females between 18 and 75 years of age, inclusive, on the day of signing informed consent 2. HbA1c ≤ 9.5% at screening 3. Diagnosis of T2D and on a stable dose of a GLP-1 receptor agonist for at least 90 days prior to screening as outlined in Table 7 4. Subjects who do not have a historical liver biopsy specimen, which is defined as a liver biopsy specimen collected ≤ 365 days prior to screening with prior confirmation of fibrosis stage 1, 2, or 3 from a pathologist, must meet inclusion criterion 4a **AND** 4b prior to collection of a liver biopsy specimen during the Screening visit: a. LSM ≥ 7.0 kPa **Note:** All subjects must complete a FibroScan® examination during the screening period, unless historical values for FibroScan® performed within 90 days prior to the Screening visit are available b. AST > 17 U/L for females and > 20 U/L for males 5. Central laboratory tests at screening that meet all of the following criteria: a. eGFR ≥ 45 mL/min, as calculated by the CKD-EPI equation b. INR ≤ 1.3, unless due to therapeutic anticoagulation c. Direct bilirubin ≤ 0.3 mg/dL d. Total bilirubin ≤ 1.3 mg/dL, unless due to an alternate etiology such as Gilbert's syndrome or hemolytic anemia e. CK < 3 × ULN

	f. Platelet count ≥ 140,000/μL g. TG level ≤ 500 mg/dL h. ALT ≤ 5 × ULN i. AST ≤ 5 × ULN j. ALP < 2 × ULN 6. Documented historical stability of ALT and AST levels, as evidenced by no significant worsening of ALT and AST values at screening relative to historical values obtained 28 days to 180 days prior to the Screening visit and based on the following parameters: a. If the historical and screening ALT and AST values are both ≤ 1.5 × ULN, there is no limit to the difference between the values b. If at least 1 of the historical or screening ALT or AST values is > 1.5 × ULN and shows worsening at screening, the percent increase must be ≤ 50% **Note:** Subjects without historical ALT and AST evaluations must have ALT and AST repeated during the screening period (Pre-baseline visit) at minimum 28 days between blood draws to confirm criterion 6a or 6b above 7. Biopsy-proven liver fibrosis due to NASH. Must have had a liver biopsy specimen collected during screening or ≤ 365 days prior to screening with confirmation of fibrosis stage 1, 2, or 3 from a pathologist 8. Subjects on Vitamin E ≥ 400 IU/day, additional antidiabetic medications (such as SGLT2 inhibitors), weight loss, or lipid-modifying medication(s) must be on a stable dose (defined as no significant change in prescription efficacy, initiation of medication, or medication discontinuation) as outlined in Table 7. A switch from one drug to another in the same class should be discussed with the Medical Monitor to confirm subject eligibility 9. Willing and able to give written informed consent prior to any study specific procedures being performed 10. Female subjects of childbearing potential (see definition in Appendix D) must have a negative serum pregnancy test at screening and a negative urine pregnancy test at baseline/Day 1

	11. Male and female subjects of childbearing potential who engage in heterosexual intercourse must agree to use protocol specified method(s) of contraception as described in Appendix D **Exclusion Criteria, Main Study** 1. Weight loss > 10% in the 90 days prior to screening until randomization or from the time of collection of the liver biopsy specimen used to assess subject eligibility until randomization, whichever is longer 2. Type 1 diabetes 3. Unstable T2D defined as: a. Insulin dose adjustment > 35% within 30 days prior to screening b. Any prior history of diabetic ketoacidosis and/or hyperglycemic hyperosmolar state 4. Hypoglycemia unawareness, hospitalization due to hypoglycemia, or history of severe hypoglycemia (hypoglycemia requiring outside assistance to regain normal neurologic status) within 90 days prior to screening 5. Subjects with osteoporosis, defined as a T-score of ≤ –2.5 at the femoral neck, total hip, or lumbar spine based on a centrally read dual-energy X-ray absorptiometry (DXA) scan performed during screening **Note:** A historical DXA scan performed within 90 days prior to screening may be accepted as the screening DXA scan. The historical scan must have been performed on a scanner previously qualified by the central imaging vendor that is available for use at post-baseline visits 6. Poorly controlled hypertension (systolic blood pressure > 160 mm Hg, or diastolic blood pressure > 100 mm Hg) at screening **Note**: Vital signs for eligibility assessment may be repeated one time at the Investigator's discretion 7. Current or prior history of decompensated liver disease requiring medical management, including ascites, hepatic encephalopathy (HE), or variceal bleeding 8. Model for End-Stage Liver Disease (MELD) score > 12, unless due to therapeutic anti-coagulation

	9. Child-Pugh score > 6 (Class B or C), unless due to therapeutic anti-coagulation 10. History of pancreatitis **Note**: Subjects with a history of gallstone pancreatitis and subsequent cholecystectomy may be allowed to participate with prior written approval from the Medical Monitor 11. Chronic hepatitis B virus (HBV) infection (hepatitis B surface antigen [HBsAg] positive) or acute hepatitis A infection (hepatitis A immunoglobulin M [IgM] antibody positive). For subjects with positive hepatitis B core antibody (HBcAb), HBV DNA by quantitative polymerase chain reaction (PCR) will be required 12. Chronic hepatitis C virus (HCV) infection (HCV antibody [Ab] and HCV RNA positive). Subjects cured of HCV infection < 2 years prior to the Screening visit (based on date of RNA PCR negative confirmation following conclusion of treatment) are not eligible. Asymptomatic subjects with spontaneous resolution of HCV infection (i.e., without RNA PCR negative confirmation following conclusion of treatment) may be allowed to participate with prior written approval from the Medical Monitor 13. Prior (< 2 years prior to screening) or planned (during the study period) bariatric surgery (e.g., gastroplasty, Roux-en-Y gastric bypass) or reversal or removal of intragastric balloon. Surgery failure < 2 years prior to screening is also exclusionary 14. Other causes of liver disease based on medical history and/or centralized review of liver histology and/or central laboratory results, including but not limited to: alcoholic liver disease, autoimmune disorders (e.g., primary biliary cholangitis [PBC], primary sclerosing cholangitis [PSC], autoimmune hepatitis), drug-induced hepatotoxicity, Wilson disease, clinically significant iron overload, or alpha-1-antitryspin deficiency 15. History of liver transplantation 16. Current or prior history of hepatocellular carcinoma (HCC) 17. Current diagnosis of Cushing's syndrome 18. History of significant alcohol consumption for a period of more than 3 consecutive months within 1 year prior to screening

	Note: Significant alcohol consumption is defined as an average exceeding 1 ethanol containing drink/day in female subjects and 2 ethanol containing drinks/day in male subjects 19. Human immunodeficiency virus (HIV) infection 20. Uncontrolled cardiac arrhythmia, or confirmed QT interval corrected using Fridericia's formula (QTcF) > 450 msec for males and > 470 msec for females at screening. Subjects with elevated QTcF (> 450 msec for males and > 470 msec for females) and a cardiac pacemaker may be allowed to participate with prior written approval from the Medical Monitor **Note**: ECG for eligibility assessment may be repeated one time at the Investigator's discretion 21. Myocardial infarction, unstable angina, percutaneous coronary intervention, coronary artery bypass graft, or stroke within 90 days prior to screening 22. Life expectancy less than 2 years 23. Use of any prohibited medication(s) as outlined in Table 5 24. Positive urine drug screen for amphetamines, cocaine, or opiates (e.g., heroin, morphine) at screening. Subjects with a positive urine drug screen due to prescription medication (e.g., opiates, methylphenidate) are eligible if the prescription and diagnosis are reviewed and approved by the Investigator. Subjects on stable methadone or buprenorphine maintenance treatment for at least 180 days prior to screening may be included in the study 25. Unable to safely undergo a liver biopsy 26. Presence of any laboratory abnormality or significant systemic or major illnesses (other than liver disease) that, in the opinion of the Investigator, compromises the subject's ability to safely participate in and complete the study including, but not limited to: a. Pulmonary disease, heart failure, renal failure, organ transplantation, serious psychiatric disease, malignancy, history of substance abuse and/or a psychiatric condition requiring hospitalization and/or emergency room visit within 180 days prior to screening 27. Unavailable for follow-up assessment or concern for subject's compliance with the protocol procedures

	28. Known hypersensitivity to the study drug, the metabolites, or formulation excipients **Exclusion Criteria, Cohort D** 1. Weight loss > 5% in the 90 days prior to screening until randomization 2. Presence of cirrhosis on liver biopsy (fibrosis stage 4) 3. Type 1 diabetes 4. Unstable T2D defined as: a. Insulin dose adjustment > 35% within 30 days prior to screening through randomization b. Any history of diabetic ketoacidosis, hyperglycemic hyperosmolar state, and/or acutely decompensated blood glucose control (e.g., thirst, polyuria, weight loss) within 90 days prior to screening 5. Hypoglycemia unawareness, hospitalization due to hypoglycemia, or history of severe hypoglycemia (hypoglycemia requiring outside assistance to regain normal neurologic status) within 90 days prior to screening 6. Subjects with osteoporosis, defined as a T-score of ≤ −2.5 at the femoral neck, total hip, or lumbar spine based on a centrally read DXA scan performed during screening **Note:** A historical DXA scan performed within 90 days prior to screening may be accepted as the screening DXA scan. The historical scan must have been performed on a scanner previously qualified by the central imaging vendor 7. Poorly controlled hypertension (systolic blood pressure > 160 mm Hg, or diastolic blood pressure > 100 mm Hg) at screening **Note:** Vital signs for eligibility assessment may be repeated one time at the Investigator's discretion 8. Current or prior history of decompensated liver disease, including ascites, HE, or variceal bleeding 9. History of pancreatitis **Note:** Subjects with a history of gallstone pancreatitis and subsequent cholecystectomy may be allowed to participate with prior written approval from the Medical Monitor

	10. Chronic HBV infection (HBsAg positive) or acute hepatitis A infection (hepatitis A IgM antibody positive). For subjects with positive HBcAb, HBV DNA by quantitative PCR will be required 11. Chronic HCV infection (HCV Ab and HCV RNA positive). Subjects cured of HCV infection < 2 years prior to the Screening visit (based on date of RNA PCR negative confirmation following conclusion of treatment) are not eligible. Asymptomatic subjects with spontaneous resolution of HCV infection (i.e., without RNA PCR negative confirmation following conclusion of treatment) may be allowed to participate with prior written approval from the Medical Monitor 12. Prior (< 2 years prior to screening) or planned (during the study period) bariatric surgery (e.g., gastroplasty, Roux-en-Y gastric bypass) or reversal or removal of intragastric balloon. Surgery failure < 2 years prior to screening is also exclusionary 13. Other causes of liver disease based on medical history and/or centralized review of liver histology and/or central laboratory results, including but not limited to: alcoholic liver disease, autoimmune disorders (e.g., PBC, PSC, autoimmune hepatitis), drug induced hepatotoxicity, Wilson disease, clinically significant iron overload, or alpha-1-antitryspin deficiency 14. History of liver transplantation 15. Current or prior history of HCC 16. History of significant alcohol consumption for a period of more than 3 consecutive months within 1 year prior to screening **Note:** Significant alcohol consumption is defined as an average exceeding 1 ethanol containing drink/day in female subjects and 2 ethanol containing drinks/day in male subjects 17. HIV infection 18. Uncontrolled cardiac arrhythmia, or confirmed QT interval corrected using Fridericia's formula (QTcF) > 450 msec for males and > 470 msec for females at screening. Subjects with elevated QTcF (> 450 msec for males and > 470 msec for females) and a cardiac pacemaker may be allowed to participate with prior written approval from the Medical Monitor **Note:** ECG for eligibility assessment may be repeated one time at the Investigator's discretion

	19. Myocardial infarction, unstable angina, percutaneous coronary intervention, coronary artery bypass graft, or stroke within 90 days prior to screening 20. Life expectancy less than 2 years 21. Use of any prohibited medication(s) as outlined in Table 5 22. Positive urine drug screen for amphetamines, cocaine, or opiates (e.g., heroin, morphine) at screening. Subjects with a positive urine drug screen due to prescription medication (e.g., opiates, methylphenidate) are eligible if the prescription and diagnosis are reviewed and approved by the Investigator. Subjects on stable methadone or buprenorphine maintenance treatment for at least 180 days prior to screening may be included in the study 23. Unable to safely undergo a liver biopsy 24. Any contraindication to magnetic resonance imaging (MRI) including, but not limited to: unmanageable claustrophobia, certain metal implants, and inability to fit in MRI machine 25. Presence of any laboratory abnormality or significant systemic or major illnesses (other than liver disease) that, in the opinion of the Investigator, compromises the subject's ability to safely participate in and complete the study including, but not limited to: a. Pulmonary disease, heart failure, renal failure, retinopathy, organ transplantation, serious psychiatric disease, malignancy, history of substance abuse and/or a psychiatric condition requiring hospitalization and/or emergency room visit within 180 days prior to screening 26. Unavailable for follow-up assessment or concern for subject's compliance with the protocol procedures 27. Known hypersensitivity to the study drug, the metabolites, or formulation excipients
Investigational Product, Dosage, and Mode of Administration:	Efruxifermin is an IgG1Fc-fibroblast growth factor 21 (FGF21) fusion protein. Efruxifermin 50 mg, EFX 28 mg, and placebo will be provided as a frozen liquid formulation and will be administered as a subcutaneous (SC) injection in the abdomen as follows: • EFX 50 mg, QW • EFX 28 mg, QW

	• Placebo, QW
Criteria for Evaluation:	
Safety:	The safety of EFX will be assessed during the **main study** and **Cohort D** through the reporting extent of exposure, adverse events (AEs), clinical laboratory tests, electrocardiograms (ECGs), ultrasounds (**main study** only), bone mineral density (BMD; **main study** only), vital sign assessments, body weight, anti-drug antibody (ADA) and neutralizing antibody (NAb) assessments, and concomitant medication usage. An external Data Monitoring Committee (DMC) that consists of at least 1 hepatologist, 1 endocrinologist, 1 cardiologist, and 1 statistician will review the progress of the study; tracking the actual annual event rate for the outcomes endpoint. The initial DMC meeting will convene after approximately 50% of subjects in the **main study** have received at least 1 dose of study drug and have either completed the Week 12 assessments or prematurely discontinued. The DMC will review any available **Cohort D** subject data at that time. The DMC's specific activities will be defined by a mutually agreed charter, which will define the DMC's membership, conduct, and meeting schedule. Throughout the study, all treatment-emergent adverse events (TEAEs), clinical assessments, clinical laboratory parameters, and criteria for drug-induced liver injury (DILI) will be closely monitored with subjects entering close observation, as required.
Pharmacokinetics:	Blood samples for pharmacokinetic (PK) analysis will be collected at specified time points as outlined in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C) and will be used to estimate steady state PK parameters (i.e., T_{max}, C_{max}, C_{trough}, AUC_{0-t}, $t_{½}$, CL/F, Vz/F) following SC administration of EFX.
Efficacy:	**Main Study** The primary efficacy endpoint is the proportion of subjects who achieve ≥ 1 stage improvement in fibrosis (based on NASH Clinical Research Network [CRN] fibrosis score) and no worsening of steatohepatitis at Week 36. Subjects will be followed for long-term safety and exploratory evaluation of clinical outcomes for approximately 96 weeks. **Secondary Endpoints:** • Proportion of subjects who achieve NASH resolution (defined as a NAS of 0—1 for inflammation and 0 for ballooning) as

	determined by the NASH CRN criteria at Week 36 and Week 96 • Proportion of subjects who achieve NASH resolution (defined as a NAS of 0—1 for inflammation and 0 for ballooning) and ≥ 1 stage improvement in fibrosis (based on NASH CRN fibrosis score) at Week 36 and Week 96 • Proportion of subjects who achieve ≥ 1 stage improvement in fibrosis (based on NASH CRN fibrosis score) and no worsening of steatohepatitis at Week 96 • Proportion of subjects who achieve ≥ 1 stage improvement in fibrosis (based on NASH CRN fibrosis score) at Week 36 and Week 96 • Change from baseline in ELF score, three components of ELF (including amino-terminal pro-peptide of type 3 procollagen [PIIINP], hyaluronic acid [HA], and tissue inhibitor of metalloproteinase-1 [TIMP-1]), pro-peptide of type 3 procollagen (Pro-C3), liver stiffness assessed by FibroScan®, and FibroScan®-AST (FAST) score • Change from baseline in TG, total cholesterol, high-density lipoprotein cholesterol (HDL-C), non-HDL—C, and low-density lipoprotein cholesterol (LDL-C) • Change from baseline in HbA1c, C-peptide, adiponectin, insulin, and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) • Change from baseline in body weight Liver biopsies will be sent to a central laboratory and read by 2 independent pathologists in a blinded manner. Week 36 and end of treatment biopsies will be combined with baseline biopsies and read by both pathologists in a blinded, random manner (but not paired). External adjudication committees will be established to adjudicate cases that meet protocol-defined criteria for liver-related events and for cardiovascular-related events. **Cohort D** The primary endpoint is the safety and tolerability of EFX compared to placebo when added to an existing GLP-1 receptor agonist. **Secondary Endpoints:**

	• Change from baseline in liver fat content as assessed by MRI-PDFF at Week 12 • Proportion of subjects who achieve a relative reduction of at least 30% in liver fat content as measured by MRI-PDFF at Week 12 • Proportion of subjects who achieve a relative reduction of at least 50% in liver fat content as measured by MRI-PDFF at Week 12 • Proportion of subjects who achieve a relative reduction of at least 70% in liver fat content as measured by MRI-PDFF at Week 12 • Proportion of subjects who achieve normalization of liver fat (liver fat content ≤ 5%) at Week 12 • Change from baseline in ALT, AST, ALP, GGT, total bilirubin, direct bilirubin, INR, plasminogen activator inhibitor-1 (PAI-1), fibrinogen, albumin, platelets, uric acid, and MELD score • Change from baseline in ELF score, three components of ELF (including PIIINP, HA, and TIMP-1), Pro-C3, liver stiffness assessed by FibroScan®, and FAST score • Change from baseline in TG, total cholesterol, HDL-C, non-HDL—C, LDL-C, apolipoprotein B, apolipoprotein C3, and lipoprotein(a) • Change from baseline in HbA1c, C-peptide, adiponectin, insulin, and HOMA-IR • Proportion of subjects who achieve normalization of HbA1c (defined as ≥ 6.5% at baseline and < 6.5% at Week 12) • Change from baseline in body weight For a complete list of study endpoints for both the **main study** and **Cohort D**, refer to Section 2.
Sample Size Calculation:	**Main Study** The primary endpoint is the proportion of subjects who achieve ≥ 1 stage improvement in fibrosis and no worsening of steatohepatitis at Week 36. Based on results of published studies in NASH with compensated cirrhosis and results of Cohort C in the Phase 2a BALANCED study of EFX, it is estimated that approximately [redacted] of subjects on placebo and [redacted] of subjects on EFX will reach this endpoint.

	With a two-sided Cochran-Mantel-Haenszel (CMH) test for proportion difference, at a significance level of 0.05, 45 completed subjects per group will provide at least 90% power. Sample size calculations are for testing 2 proportions using a CMH test and were computed using the normal approximation method. Factoring in dropouts, the study plans to randomize at least [redacted] subjects in total ([redacted] per group). The **main study** planned to enroll [redacted] subjects, but over enrolled by approximately 20%. **Cohort D** Since the primary objective of **Cohort D** is to assess the safety and tolerability of EFX compared to placebo when added to an existing GLP-1 receptor agonist in subjects with T2D and liver fibrosis due to NASH, the sample size is determined based on clinical considerations. The study is not powered for comparisons of efficacy measurements across treatment groups.
Statistical Methods:	Detailed description of data analysis and statistical methods to be used will be outlined separately in a Statistical Analysis Plan (SAP).
Population:	The Full Analysis Set (FAS) will include all subjects who were randomized and received at least one dose of study drug. The analysis set for the histological efficacy analyses will be the Liver Biopsy Analysis Set (LBAS) which will be a subset of the FAS. The LBAS will include all FAS subjects who have baseline and on-study liver biopsy results. The analysis set for safety analyses will be the Safety Analysis Set which includes all subjects who received at least 1 dose of study drug.
Efficacy Analysis:	**Main Study** **Primary Efficacy Analysis:** A CMH test will be used to compare the differences in the proportion of subjects who achieve ≥ 1 stage improvement in fibrosis without worsening of steatohepatitis at Week 36 between the EFX arm and the placebo arm, adjusting for stratification factors. The point estimates and [redacted] confidence intervals for the differences in proportions will be calculated. **Secondary Efficacy Analysis**: For secondary endpoints, such as resolution of steatohepatitis, a stratified CMH test will be performed to compare the differences in proportions between the EFX arm and the placebo arm. For continuously variable measures of efficacy, analysis of covariance (ANCOVA) or mixed-model repeated measures (MMRM) will be used instead.

	Cohort D Efficacy Analysis: Summary statistics (number of subjects, mean, standard deviation, median, minimum, and maximum) at all visits and change from baseline will be provided. The continuous efficacy variables will be analyzed with an ANCOVA model or MMRM with treatment group and liver fibrosis stage (1 vs 2-3) as factors and baseline value as a covariate. Normality will be tested for the model residuals.
Pharmacokinetic Analysis:	Plasma concentrations of EFX will be listed and summarized.

This study will be conducted in accordance with the guidelines of Good Clinical Practice (GCP) including archiving of essential documents.

TABLE OF CONTENTS

STUDY ACKNOWLEDGEMENT SIGNATURE PAGE....2
SYNOPSIS....3
TABLE OF CONTENTS....23
LIST OF TABLES....28
LIST OF FIGURES....28
LIST OF APPENDICES....28
LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS....29
1. BACKGROUND AND RATIONALE....34

1.1. Background....34

1.1.1. Fibroblast Growth Factor 21....34

1.2. Efruxifermin Background....35
1.3. Nonclinical Pharmacology and Toxicology....36
1.4. Clinical Trials of Efruxifermin....38

1.4.1. Study AK-US-001-0101 - Phase 2a, Randomized, Double-Blind, Placebo-Controlled Study Evaluating the Safety and Efficacy of EFX in Subjects with Nonalcoholic Steatohepatitis (NASH)....38

1.5. Pharmacokinetic Results....45
1.6. Rationale for this Study....46
1.7. Rationale for Selection of Dose....48
1.8. Compliance....49

2. STUDY OBJECTIVES AND ENDPOINTS....50
3. INVESTIGATIONAL PLAN....55

3.1. Overall Study Design and Plan....55
3.2. Randomization and Treatment Assignment....57
3.3. Study Duration and Number of Centers....57
3.4. Early Termination....57
3.5. 30-day Follow-up....58
3.6. Post Study Care....58

4. STUDY POPULATION SELECTION....59

4.1. Study Population....59
4.2. Inclusion Criteria....59

4.2.1. Main Study....59
4.2.2. Cohort D....61

4.3. Exclusion Criteria....63

4.3.1. Main Study....63
4.3.2. Cohort D....65

5. STUDY DRUG(S)....69

5.1. Randomization, Blinding and Treatment Codes....69

5.1.1. Procedures for Breaking Treatment Codes....69

5.2. Efruxifermin and Placebo....69

5.2.1. Formulation, Packaging, and Labeling....69
5.2.2. Storage and Handling....70
5.2.3. Dosage and Administration....70

5.3. Prior and Concomitant Medications....71
5.4. Study Drug Accountability....76

5.4.1. Study Drug Return or Disposal....76

6. STUDY PROCEDURES....77

6.1. Study Procedures in Case of Crisis Situations....77
6.2. Screening and Informed Consent....78
6.3. Demographics and Medical History....79
6.4. Physical Examination....79
6.5. Vital Signs, Height, and Weight....80

6.5.1. Vital Signs....80
6.5.2. Height....80
6.5.3. Weight....80

6.6. Hip and Waist Circumference – Cohort D Only....80
6.7. 12-Lead Electrocardiogram....81
6.8. Model for End-Stage Liver Disease (MELD) Score....81
6.9. Child-Pugh Score....81
6.10. Abdominal Ultrasound – Main Study Only....81
6.11. FibroScan®....81
6.12. Liver Biopsy....82
6.13. Optional Magnetic Resonance Elastography (MRE) Substudy – Main Study Only....83
6.14. Magnetic Resonance Imaging - Proton Density Fat Fraction (MRI-PDFF) – Cohort D Only....84
6.15. Dual-energy X-ray Absorptiometry (DXA) Scan....84
6.16. Chronic Liver Disease Questionnaire-Nonalcoholic Steatohepatitis (CLDQ-NASH) – Main Study Only....84
6.17. Work Productivity and Activity Impairment for Nonalcoholic Steatohepatitis (WPAI-NASH) – Main Study Only....85

6.18. Lifestyle Guidance 85
6.19. Laboratory Assessments 86

6.19.1. Laboratory Sample Collection, Storage, and Shipping 86
6.19.2. Pharmacokinetic Assessments 88
6.19.3. Optional Genetic Substudy 89
6.19.4. Optional Peripheral Blood Mononuclear Cell Substudy – Main Study Only 89
6.19.5. Immunogenicity Assessments 89
6.19.6. Biomarkers 89
6.19.7. Sample Storage 90

6.20. Subject Retention 90
6.21. Criteria for Discontinuation of Study Drug 91
6.22. Interruption of Study Drug 92
6.23. Adverse Events and Toxicity Management 92

6.23.1. Adverse Events 92
6.23.2. Study Stopping Rules 94
6.23.3. Overdose 94
6.23.4. Serious Adverse Events 95
6.23.5. Reporting Adverse Events 95
6.23.6. Adverse Events of Special Interest 98
6.23.7. Clinical Laboratory Abnormalities and Other Abnormal Assessments as Adverse Events or Serious Events 99
6.23.8. Adjudication Committees 100
6.23.9. Observation for Drug-Induced Liver Injury (DILI) 101
6.23.10. Removal of Subjects from the Study 105

7. PLANNED STATISTICAL METHODS 107

7.1. Endpoints 107

7.1.1. Efficacy Endpoints 107
7.1.2. Safety Endpoints 107

7.2. Planned Analyses 107

7.2.1. Primary Endpoint Analysis – Main Study Only 107
7.2.2. Final Analysis 108

7.3. Analysis Conventions 108
7.4. Analysis Sets 108

7.4.1. Full Analysis Set (FAS) 108
7.4.2. Per Protocol (PP) Sets 108
7.4.3. Safety Analysis Set 108
7.4.4. Pharmacokinetics Set 108

7.4.5. Biomarkers Analysis Set....108
7.4.6. Liver Biopsy Analysis Set (LBAS) – Main Study Only....109
7.4.7. MRI-PDFF Analysis Set – Cohort D Only....109

7.5. Data Handling Conventions....109
7.6. Estimands – Main Study Only....109
7.7. Demographic Data and Baseline Characteristics....110
7.8. Efficacy Analysis....110

7.8.1. Primary Efficacy Analysis – Main Study Only....110
7.8.2. Secondary Efficacy Analysis – Main Study Only....111
7.8.3. Exploratory Efficacy Analysis – Main Study Only....111
7.8.4. Cohort D Efficacy Analyses....111

7.9. Safety Analysis....111

7.9.1. Extent of Exposure....111
7.9.2. Adverse Events....112
7.9.3. Laboratory Evaluations....112

7.10. Pharmacokinetic Analysis....112
7.11. Biomarker Analysis....113
7.12. Immunogenicity Analysis....113
7.13. Sample Size....113
7.14. Data Monitoring Committee....113

8. ADMINISTRATIVE CONSIDERATIONS....115

8.1. Investigator Responsibilities....115

8.1.1. Good Clinical Practice....115
8.1.2. Institutional Review Board (IRB)/Independent Ethics Committee (IEC) Review and Approval....115
8.1.3. Informed Consent....115
8.1.4. Confidentiality....116
8.1.5. Study Files and Retention of Records....116
8.1.6. Electronic Case Report Forms....117
8.1.7. Inspections....118
8.1.8. Protocol Compliance....118

8.2. Sponsor Responsibilities....118

8.2.1. Protocol Modifications....118
8.2.2. Study Report and Publications....118

8.3. Joint Investigator/Sponsor Responsibilities....119

8.3.1. Payment Reporting....119
8.3.2. Access to Information for Auditing or Inspections....119
8.3.3. Study Discontinuation....119

9. REFERENCE LIST....120
10. APPENDICES....124

LIST OF TABLES

Table 1. Summary of Week 12 Efficacy Endpoints (Full Analysis Set) 40
Table 2. Summary of Histology Endpoints (Liver Biopsy Analysis Set).....41
Table 3. Main Study Objectives and Endpoints.....50
Table 4. Cohort D Study Objectives and Endpoints 53
Table 5. Prohibited Concomitant Medications 71
Table 6. Main Study Conditionally Allowed Medications 73
Table 7. Cohort D Conditionally Allowed Medications 75
Table 8. Laboratory Assessments 87
Table 9. Optional Pharmacokinetic Substudy – Day 1 88
Table 10. Optional Pharmacokinetic Substudy – Week 6 to Week 12 89
Table 11. Grading of Adverse Event Not Covered by the CTCAE 93
Table 12. Approach for Intercurrent Events for the Primary Endpoint 110
Table 13. Child-Pugh Score Components.....139
Table 14. Definition of Terms.....140

LIST OF FIGURES

Figure 1-1. Efruxifermin Schematic Structure with Disulfide Bonds and Constituent Polypeptide Chains 36
Figure 3-1. Study Design – Main Study 55
Figure 3-2. Study Design – Cohort D.....56
Figure 6-1. Considerations for Close Observation 102
Figure 6-2. Considerations for Study Drug Interruption 103

LIST OF APPENDICES

Appendix A. Main Study Schedule of Assessments Table – Screening through Week 36 125
Appendix B. Main Study Schedule of Assessments Table – Long-Term Follow-up.....129
Appendix C. Cohort D Schedule of Assessments Table 132
Appendix D. Pregnancy Precautions, Definition for Female of Childbearing Potential, and Contraceptive Requirements 135
Appendix E. West Haven Criteria.....138
Appendix F. Child-Pugh Score 139
Appendix G. Definitions of Compensated Cirrhosis Due to Nonalcoholic Steatohepatitis (NASH) 140

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AAT	alpha-1-antitrypsin
Ab	antibody
ADA	anti-drug antibodies
ADL	activities of daily living
AE	adverse event
AESI	adverse event of special interest
ALP	alkaline phosphatase
ALT	alanine aminotransferase (serum glutamate pyruvate transaminase)
ANCOVA	analysis of covariance
AST	aspartate aminotransferase (serum glutamate oxaloacetate transaminase)
AUC	area under the time-concentration curve
BMD	bone mineral density
BMI	body-mass index
BSAP	bone specific alkaline phosphatase
C3M	type III collagen
CAP	controlled attenuation parameter
CFR	code of federal regulations
CK	creatine kinase
CKD-EPI	chronic kidney disease epidemiology collaboration
CLDQ-NASH	chronic liver disease questionnaire-nonalcoholic steatohepatitis
C_{max}	maximum plasma drug concentration
CMH	cochran-mantel-haenszel
CRF	case report form
CRN	clinical research network
CRO	clinical research organization
CSR	clinical study report
CTCAE	common terminology criteria for adverse events
CTX-1	c-telopeptide of type I collagen
DILI	drug-induced liver injury
DMC	data monitoring committee

DNA	deoxyribonucleic acid
DXA	dual-energy x-ray absorptiometry
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
EFS	event-free survival
EFX	efruxifermin
eGFR	estimated glomerular filtration rate
ET	early termination
EIU	exposure in utero
ELF™	enhanced liver fibrosis panel
EU	european union
FAS	full analysis set
FAST	fibroscan® aspartate aminotransferase score
FDA	food and drug administration
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
FSH	follicle-stimulating hormone
GCP	good clinical practice
GGT	gamma glutamyl transferase
GLP-1	glucagon-like peptide-1 (agonist)
HA	hyaluronic acid
HbA1c	hemoglobin A1c
HBcAb	hepatitis B core antibody
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDL-C	high-density lipoprotein cholesterol
HE	hepatic encephalopathy
HIV	human immunodeficiency virus
HLGT	high-level group term

HLT	high-level term
HOMA-IR	homeostasis model assessment of insulin resistance
IB	investigator's brochure
ICF	informed consent form
ICH	international council for harmonization
IEC	independent ethics committee
IgG1	human immunoglobulin g1
IgM	immunoglobulin M
IND	investigational new drug
INR	international normalized ratio
IQR/M	interquartile range/median value
IRB	institutional review board
IRT	interactive response technology
ISO	international organization for standardization
kPa	kilopascals
LBAS	liver biopsy analysis set
LDL-C	low-density lipoprotein cholesterol
LLT	low-level term
LOCF	last observation carried forward
LS	least-squares
LSM	liver stiffness measurement
MedDRA	medical dictionary for regulatory activities
MELD	model for end-stage liver disease
MMRM	mixed-model repeated-measures
MRE	magnetic resonance elastography
MRI	magnetic resonance imaging
MRI-PDFF	magnetic resonance imaging – proton density fat fraction
NAFLD	non-alcoholic fatty liver disease
NAb	neutralizing antibody
NAS	non-alcoholic fatty liver disease activity score
NASH	nonalcoholic steatohepatitis
NOAEL	no-observed-adverse-effect-level

NRS	numeric rating scale
P1NP	procollagen type 1 N pro-peptide
PIIINP	pro-peptide of type 3 procollagen
PBC	primary biliary cholangitis
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PEth	phosphatidylethanol
PK	pharmacokinetics
PP	per protocol
PNPLA3	patatin-like phospholipase domain-containing protein 3
Pro-C3	pro-peptide of type 3 procollagen
PSC	primary sclerosing cholangitis
PT	preferred term
QTcF	QT interval corrected using Fridericia's formula
QW	once weekly
RNA	ribonucleic acid
SC	subcutaneous
SAE	serious adverse event
SAP	statistical analysis plan
SAS®	statistical analysis system®
SGLT2	sodium glucose co-transporter 2
SOC	system organ class
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reaction
$t_{1/2}$	time to half plasma concentration (half-life)
T2D	type 2 diabetes
TBL	total bilirubin
TEAEs	treatment-emergent adverse events
TESAEs	treatment-emergent serious adverse events
TG	triglyceride
TIMP-1	tissue inhibitor of metalloproteinases 1
T_{max}	time to maximum plasma concentration

ULN	upper-limit of normal
US	united states
VLDL-C	very low-density lipoprotein cholesterol
WPAI-NASH	work productivity and activity impairment for nonalcoholic steatohepatitis

1. BACKGROUND AND RATIONALE

1.1. Background

Nonalcoholic steatohepatitis (NASH) is the most severe form of non-alcoholic fatty liver disease (NAFLD) and is characterized by the presence of an abnormal accumulation of fat in the liver, which in some individuals can progress to liver cell injury (hepatocellular ballooning), inflammation, and ultimately fibrosis. Lipotoxicity and oxidative stress are considered to be the drivers of disease progression, or the underlying cause of the disease. As NASH progresses, it can result in excessive deposition of extracellular collagen, or scarring of the liver (fibrosis), ultimately leading to liver cirrhosis. Later stages of NASH are associated with substantially increased risk of hepatocellular carcinoma (HCC) and major adverse cardiac events (Ekstedt et al. 2015), underlining the healthcare burden presented by NASH in the absence of approved therapies.

Nonalcoholic steatohepatitis is closely related to the epidemic of obesity and diabetes and is often viewed as the liver manifestation of the metabolic syndrome. It is heavily influenced by lifestyle (e.g., chronically excessive calorie intake, sedentary lifestyle) and is distinct from other fatty liver diseases caused by viral infection, alcohol abuse, or medication side effects (EASL et al. 2016). Nonalcoholic steatohepatitis can potentially progress to advanced liver disease, cirrhosis, and HCC (The Nash Education Program 2019). The current prevalence of NASH in the United States (US) population is estimated to be around 3–4%. Approximately 1 in 5 of the nearly 80 million patients with NAFLD in the US (Ruhl & Everhart 2015) are projected to develop NASH (Younossi et al. 2016). This emerging epidemic liver disease warrants a major public health effort to control the burden of disease, in particular its significant complications and comorbid conditions.

1.1.1. Fibroblast Growth Factor 21

Fibroblast growth factor 21 (FGF21) is a member of the endocrine fibroblast growth factor (FGF) sub-family. Fibroblast growth factor 21 forms a ligand/co-receptor complex by first anchoring to β-Klotho on the cell surface, then interacting with 1 of 3 members of the fibroblast growth factor receptor (FGFR) family (specifically FGFR1c, 2c and 3c) to induce dimerization and autophosphorylation of FGFR. Activated FGFR initiates specific intracellular signaling cascades leading to the expression of FGF21's paracrine and endocrine biological functions (Li et al. 2013). Fibroblast growth factor 21 is a secreted polypeptide highly expressed in tissues relevant to metabolic function, including the liver, adipose tissue, and pancreas (Fon Tacer et al. 2010, Nishimura et al. 2000). Studies have shown that FGF21 is a metabolic regulator of energy homeostasis, maintaining appropriate balance across glucose-lipid-protein utilization, and enhancing insulin sensitivity in the fed state (Kim et al. 2017, Gaich et al. 2013, Li et al. 2013, Talukdar et al. 2016, Zhao et al. 2019). Fibroblast growth factor 21 directly modulates whole-body lipid metabolism to reduce hepatic lipid accumulation. Fibroblast growth factor 21 has been shown to reverse hepatic steatosis and to prevent diet-induced obesity in both rodents and nonhuman primates (Lin et al. 2013, Camporez et al. 2013, Mu et al. 2012, Xu et al. 2009, Coskun et al. 2008, Wu et al. 2017, Talukdar et al. 2016). Hence, FGF21 is a target for the

development of novel biological drugs for metabolic diseases (Gimeno & Moller 2014, Chen et al. 2017). Serum FGF21 levels are elevated in human subjects with diabetes, obesity, NASH, and other metabolic disorders. As such, raised levels reflect increased secretion of FGF21 by organs and cells under various types of stress (e.g., nutrient stress, oxidative stress) and appears to be a compensatory protective response. Higher levels of FGF21 have also been interpreted as evidence of FGF21 resistance (Li et al. 2013, Wu et al. 2017).

In NASH, FGF21 appears to address both the underlying disease driver, i.e., excessive flux of calories and lipids into the liver, and the ensuing downstream sequelae of hepatocyte stress leading to hepatic inflammation and fibrosis. Fibroblast growth factor 21 directly modulates whole-body lipid metabolism to reduce hepatic lipid accumulation and has been shown to reverse hepatic steatosis and to prevent diet-induced obesity in both rodents and nonhuman primates (Lin et al. 2013, Camporez et al. 2013, Mu et al. 2012, Xu et al. 2009, Coskun et al. 2008, Wu et al. 2017, Talukdar et al. 2016).

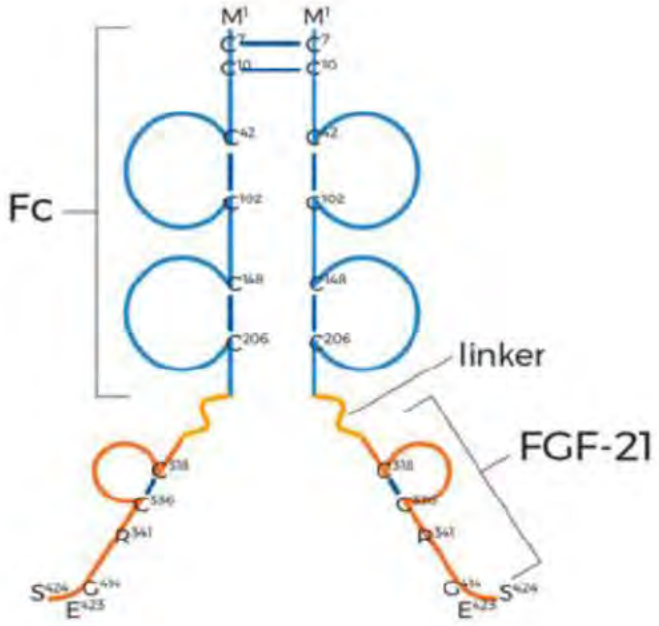
Various in vitro and in vivo findings suggest potential beneficial effects of FGF21 agonism in NASH, including improvements in liver fat, inflammation, and fibrosis (Xu et al. 2009, Lee et al. 2016, Tanaka et al. 2015, Cui et al. 2020). Administration of recombinant human FGF21 or long-acting FGF21 analogues prevented an increase in liver fat and suppressed inflammation and fibrosis, as assessed by histology in rodent models of liver disease (Tanaka et al. 2015, Fisher et al. 2014, Bao et al. 2018, Opoku et al. 2020). The models used a range of pathological insults, including excessive intake of fat and fructose, alcohol, diet deficient in methionine and choline, or chemical toxins (e.g., carbon tetrachloride, dimethyl nitrosamine; [Xu et al. 2016]). Increasing FGF21 tone was effective in all the models, whereas loss of FGF21 function exacerbated development of liver pathology.

Genetic and pharmacological studies provide further evidence that FGF21-receptor agonism could have potentially beneficial effects on NASH. Fibroblast growth factor 21 knock-out mice are viable, fertile, and show no strong metabolic phenotypes when fed a normal chow diet. However, they become insulin resistant with hepato-steatosis and have significantly elevated plasma glucose and triglyceride (TG) levels when challenged with high-fat or ketogenic diets. In contrast, transgenic mice over-expressing FGF21 are lean and resistant to diet-induced or age-associated obesity and insulin resistance.

1.2. Efruxifermin Background

Efruxifermin (EFX) is a 92.0 kD *E. coli*-expressed human immunoglobulin G1 (IgG_1) Fc-FGF21 fusion polypeptide—a long-acting FGF21 analog. Each molecule contains 1 dimeric Fc domain and 2 modified FGF21 chains. Efruxifermin has 8 disulfide bonds, 6 intra-chain and 2 inter-chain, as depicted in Figure 1-1. Two of the intra-chain disulfide bonds are in the FGF21 polypeptide between Cys318 and Cys336; 1 for each monomer. Additionally, 3 mutation sites, L98R (corresponding residue 341 in Fc-FGF21 fusion polypeptide), P171G (residue 414), and A180E (residue 423) have been introduced into the consensus human FGF21 sequence to decrease its susceptibility to in vivo proteolytic degradation, and to reduce aggregation while maintaining balanced FGF21 agonism across FGFR1c/2c/3c with selectivity over FGFR4. A schematic of the structure of EFX is shown in Figure 1-1.

Figure 1-1. Efruxifermin Schematic Structure with Disulfide Bonds and Constituent Polypeptide Chains



1.3. Nonclinical Pharmacology and Toxicology

In vitro characterization of EFX demonstrated high affinity binding (< 10nM) to human, cynomolgus monkey, and mouse β-Klotho. Efruxifermin was demonstrated to activate c-isoforms of FGFR: FGFR1c, 2c and 3c, with balanced potency and high selectivity over FGFR4 using a cell-line co-expressing human β-Klotho and each FGFR individually (Stanislaus et al. 2017).

In vivo, EFX is pharmacologically active in rodents and monkeys. Administration of EFX to obese and insulin resistant mouse and monkey models improved the metabolic status of these animals resulting in reduced plasma glucose, insulin, and TG levels; improved lipoprotein profile; enhanced glucose tolerance; and significant weight loss (Stanislaus et al. 2017).

Efruxifermin has been evaluated in 16- and 26-week nonclinical toxicology studies in rats and monkeys administered subcutaneous (SC) doses of 0 (vehicle control), 10, 30, or 100 mg/kg, once weekly (QW). Effects observed in these studies in both species included decreases in body weight gain/body weight, decreases in lean body mass and body fat, decreased bone growth secondary to effects on body weight, and minimal increases in alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) without concomitant liver pathology. Increased adipocyte cellularity in bone marrow was observed in rats. These effects were consistent with and attributed to the known pharmacological effects of FGF21 analogs such as EFX. Decreased red cell mass (erythrocytes, hemoglobin, and/or hematocrit), decreased thymic lymphoid cellularity, decreases in reproductive organ weights in both sexes, and minimal to mild atrophy of uterus, cervix, and vagina in monkeys were attributed to stress and/or reduced body

weight/growth. Efruxifermin-related changes were generally reversible or exhibited a trend towards reversibility in both species. The no adverse effect level (NOAEL) in both rat studies was 100 mg/kg/dose; in the 26-week study, associated C_{max} values at the NOAEL were approximately 9- to 19-fold the modeled C_{max} in humans at EFX 50 mg, and corresponding $AUC_{0\text{-}96h}$ values were approximately 20- to 41-fold higher than the modeled $AUC_{0\text{-}168h}$ in humans at EFX 50 mg. The NOAEL in the 16-week monkey study was 30 mg/kg/dose. The NOAEL in the 26-week monkey study was 30 mg/kg/dose in females, and not identified in males due to overall inhibition of growth with resulting deficit in bone growth and consolidation. In the 26-week monkey study, the C_{max} value at the NOAEL was 27-fold higher than the modeled C_{max} in humans at EFX 50 mg, and corresponding $AUC_{0\text{-}96h}$ value was 11-fold higher than the modeled $AUC_{0\text{-}168h}$ in humans at EFX 50 mg. The exposure multiples based on area under the drug concentration versus time curve (AUC) values are considered conservative based on values calculated through 96 hours post-dose in animals compared to modeled values through 168 hours post-dose in humans.

Genotoxicity studies have not been conducted to date with EFX. A 2-year rat carcinogenicity study evaluating 1, 3, and 10 mg EFX/kilogram body weight/per week is in progress with results anticipated in 2024. However, studies have been conducted to examine the potential of EFX to impact cell proliferation and mitogenicity. Results of in vitro and short-term in vivo studies demonstrated that EFX was not a direct-acting mitogen and was not associated with persistent cell proliferation in the liver. Moreover, staining of the liver with Ki67, a marker of active cell proliferation, showed no changes after 26 weeks of repeat-dosing of rats QW at up to 100 mg/kg. In addition, there were no pre-neoplastic histopathology changes observed in rats or in monkeys administered weekly SC doses up to 100 mg/kg/dose for 26 weeks. These data suggest that EFX is expected to have minimal risk for carcinogenicity.

In fertility and early embryonic development studies in rats, EFX administration to females prior to and during mating did not impair fertility or numbers of embryos at doses up to 30 mg/kg, nor did it impair fertility of males at doses up to 100 mg/kg. In embryofetal development studies in rats and rabbits administered EFX every 3 days during gestation, no maternal toxicity was observed up to 100 mg/kg/dose and no embryofetal toxicity occurred at doses up to 30 mg/kg/dose. Efruxifermin was not teratogenic in rats or rabbits.

Refer to the EFX Investigator's Brochure (IB) for additional details.

1.4. Clinical Trials of Efruxifermin

As of 01 May 2021, EFX has been evaluated in Phase 1 single- and multiple-ascending dose studies with up to a 4-week treatment duration in subjects with type 2 diabetes (T2D), a Phase 2a study in subjects with NASH that comprised of 2 cohorts (fibrosis stages 1—3 and a separate cohort of subjects with fibrosis stage 4), and it is currently being evaluated in a Phase 2b study in non-cirrhotic subjects with NASH (fibrosis stages 2—3). In the Phase 1a single-ascending dose study, the highest dose tested was EFX 210 mg administered SC. In the Phase 1b multiple-ascending dose study, the highest dose tested was EFX 140 mg, SC, QW. In the Phase 2a study, 28, 50, and 70 mg doses of EFX were evaluated over 16-weeks of treatment. In all studies, the drug was generally well tolerated. Findings from both cohorts of the Phase 2a study are briefly described below. Information on the Phase 1 clinical studies and additional details on the Phase 2 clinical study are described in further detail in the current EFX IB.

1.4.1. Study AK-US-001-0101 - Phase 2a, Randomized, Double-Blind, Placebo-Controlled Study Evaluating the Safety and Efficacy of EFX in Subjects with Nonalcoholic Steatohepatitis (NASH)

Study AK-US-001-0101 was a Phase 2a, randomized, double-blind, placebo-controlled study evaluating the safety and efficacy of EFX in subjects with NASH. The study consisted of a Main Study in subjects with fibrosis stages 1—3 (Section 1.4.1.1) and a separate cohort, Cohort C, in subjects with compensated cirrhosis or fibrosis stage 4 (Section 1.4.1.2).

1.4.1.1. Main Study

The primary endpoint was to evaluate the absolute change from baseline in hepatic fat fraction assessed by Magnetic Resonance Imaging - Proton Density Fat Fraction (MRI-PDFF) at Week 12. Secondary and exploratory endpoints included evaluation of percent change in hepatic fat fraction assessed by MRI-PDFF, histology, reductions in ALT, and safety and tolerability.

1.4.1.1.1. Subject Disposition

In the Main Study, eligible subjects were randomized to receive 28, 50, or 70 mg of EFX or placebo in a ratio of 1:1:1:1. The planned sample size was approximately 80 subjects. Efuxifermin or placebo was administered QW for 16 weeks.

A total of 80 subjects were enrolled with 79 (19 [24.1%], 19 (24.1%), and 20 (25.3%) subjects in the 28-, 50-, and 70-mg EFX groups, respectively, and 21 (26.6%) subjects in the placebo group) subjects receiving at least 1 dose of study drug. A total of 66 (82.5%) subjects completed investigational product as per the dosing regimen for 16 weeks. Thirteen (16.3%) subjects discontinued investigational product (3, 4, and 4 subjects in the 28-, 50-, and 70-mg EFX groups, respectively, and 2 subjects in the placebo group). Seven (9.0%) subjects discontinued investigational product because of adverse events (AEs) and 7 (9.0%) subjects discontinued the study due to administrative reasons. A total of 66 (82.5%) subjects completed the study.

1.4.1.1.2. Safety Results

Overall, 70 (88.6%) subjects experienced a treatment-emergent adverse event (TEAE): 18 (94.7%), 17 (89.5%), and 19 (95.0%) subjects in the 28-, 50-, and 70-mg EFX groups, respectively, and 16 (76.2%) subjects in the placebo group. The most common TEAEs by preferred term (PT) were diarrhea (27 [34.2%] subjects), nausea (26 [32.9%] subjects), vomiting and increased appetite (15 [19.0%] subjects each), fatigue (13 [16.5%] subjects), and nasopharyngitis (11 [13.9%] subjects).

The majority of subjects experienced a Grade 1 or 2 TEAE with the maximum TEAE severity being Grade 1 in 18 (22.8%) subjects, Grade 2 in 46 (58.2%) subjects, and Grade 3 in 5 (6.3%) subjects. One (1.3%) subject (Subject 101-006 in the 70-mg EFX group) experienced a Grade 4 treatment-emergent serious adverse event (TESAE) of acute pancreatitis (considered related to study drug) and subsequently, diabetic ketoacidosis (considered unrelated to study drug).

Fifty-two (65.8%) subjects experienced a study drug-related TEAE: 13 (68.4%), 15 (78.9%), and 17 (85.0%) subjects in the 28-, 50-, and 70-mg EFX groups, respectively, and 7 (33.3%) subjects in the placebo group. The majority of study drug-related TEAEs were Grade 1 or 2.

One (5.0%) subject experienced a TESAE of acute pancreatitis in the 70-mg EFX group that was considered related to study drug. There were no study drug-related deaths due to TEAEs.

Overall, 2 (2.5%) subjects experienced a serious adverse event (SAE): 1 (5.3%) subject in the 28-mg EFX group experienced abdominal pain and pyrexia, which was considered unrelated to the study drug but related to the pre-treatment liver biopsy procedure; 1 (5.0%) subject in the 70-mg EFX group experienced acute pancreatitis which was deemed to be related to study drug and subsequently, diabetic ketoacidosis. This subject was morbidly obese (body-mass index [BMI] 46.6 kg/m^2) and had multiple metabolic abnormalities, including severe insulin resistance as indicated by acanthosis nigricans and hyperinsulinemia prior to starting the study. Following withdrawal of study drug and appropriate management of acute pancreatitis, including hospitalization and administration of medications, the subject recovered, was discharged from the hospital, and the event was considered resolved.

In the subjects receiving at least 1 dose of EFX and considered evaluable for the assessment of a treatment-emergent anti-drug antibody (ADA) response, 41 of 57 individuals (71.9%) were positive for treatment-induced ADA, compared to none in the placebo group. While the majority of subjects developed ADA during the study, individual data from all subjects with treatment-induced ADA indicated that the measured antibody titers were low with a median observed titer value at 20 weeks of 1:8.67. In addition, only 4 subjects (7.0% of EFX-treated subjects) demonstrated emergence of FGF21 cross-reactive antibodies. Only 1 subject was positive for neutralizing antibodies (NAb) at a single time point, and this subject subsequently tested negative for ADA.

1.4.1.1.3. Efficacy Results

All 3 EFX dose groups for the Full Analysis Set (FAS) met the primary and secondary endpoints, demonstrating statistically significant reductions in liver fat and ALT at Week 12 (Table 1). The EFX treatment groups demonstrated a statistically significant reduction in absolute change from baseline (reduction) in hepatic fat fraction of 12.3%, 13.4% and 14.1% in the 28-, 50-, and 70-mg EFX treatment groups, respectively ($p<0.0001$ for each). Among subjects receiving placebo, a 0.3 reduction ($p = 0.3828$) was observed.

All 3 EFX treatment groups demonstrated a statistically significant relative reduction of hepatic fat fraction from baseline to Week 12 of 63.2%, 70.9% and 72.3% respectively for subjects receiving 28, 50 and 70 mg of EFX ($p<0.0001$ for each dose group vs. baseline), while a 0.3% reduction was observed for placebo subjects ($p = 0.9494$).

As detailed in Table 1, a large proportion of EFX-treated subjects were "responders" as defined by a ≥ 30% reduction in hepatic fat fraction at Week 12. In the 28-mg, 50-mg, and 70-mg EFX treatment groups, respectively, 84.2%, 85.0% and 75.0% of subjects were responders compared to 9.5% in the placebo group ($p<0.0001$ or $p<0.0002$ when compared to placebo).

When analyzing the MRI-PDFF Evaluable Analysis Set (defined as subjects who had baseline and Week 12 hepatic fat fraction assessed by MRI-PDFF), 16 (100.0%), 17 (100.0%), and 15 (100.0%) subjects in the 28-, 50-, and 70-mg EFX groups, respectively, and 2 (10.0%) subjects in the placebo group had a ≥ 30% reduction at Week 12. Moreover, 68.8%, 100.0%, and 93.3% of subjects in the 28-, 50-, and 70-mg EFX groups experienced a ≥ 50% reduction in hepatic fat fraction compared to only 5.0% in the placebo group.

A statistically significant reduction in ALT least-squares (LS) mean values from baseline to Week 12 was also observed in EFX-treated subjects ($p<0.0001$ for each dose group vs. baseline): 24.5 U/L, 30.5 U/L and 32.3 U/L in the 28-, 50- and 70-mg groups, respectively.

Table 1. Summary of Week 12 Efficacy Endpoints (Full Analysis Set)

Measure	Placebo (N=21)	EFX (once weekly dose)		
		28 mg (N=19)	50 mg (N=20)	70 mg (N=20)
Absolute reduction in hepatic fat fraction[a] (%)	−0.3	−12.3[d]	−13.4[d]	−14.1[d]
Normalization (< 5%) of hepatic fat fraction[b] (%)	5	21	45	50[c]
Relative reduction in hepatic fat fraction[a] (%)	0	−63[d]	−71[d]	−72[d]
≥ 30% Relative reduction in hepatic fat fraction[b] (%)	10	84[d]	85[d]	75[d]
≥ 50% Relative reduction in hepatic fat fraction[b] (%)	5	58[c]	85[d]	70[d]
Reduction in ALT[a] (U/L)	−6	−24[d]	−30[d]	−32[d]

ALT = alanine aminotransferase; EFX = Efruxifermin
a Least-squares mean change from baseline
b Least-squares mean proportion of subjects
c $p<0.004$, versus placebo
d $p<0.001$, versus placebo

Subjects who achieved ≥ 30% reduction in hepatic fat fraction after 12 weeks of treatment were eligible for a biopsy after 16 weeks of treatment. The liver biopsy analysis set included all responders who had baseline and end-of treatment liver biopsy results. Biopsies were obtained from 2, 13, 13, and 14 subjects respectively for the placebo group and the 28-mg, 50-mg, and 70-mg EFX treatment groups. Due to COVID-19 and other subject-specific issues, biopsies could not be obtained from 8 additional biopsy-eligible subjects, who were all in EFX treatment groups.

A responder based on NAFLD Activity Score (NAS) was defined as a subject with a decrease of ≥ 2 points in NAS with at least a 1-point reduction in either lobular inflammation or hepatocellular ballooning with no concurrent worsening of fibrosis stage. Results for proportion of subjects who were NAS responders and for other histologic improvements are provided in Table 2.

Of the 40 EFX-treated subjects, a total of 11 (27.5%) achieved ≥ 2 stage improvement in fibrosis regression.

Resolution of NASH (subjects who had a score of 0—1 for inflammation and 0 for ballooning) without worsening of fibrosis stage was achieved by 19 (47.5%) of the 40 EFX-treated subjects overall. One of 2 placebo subjects met this endpoint.

Across the 40 EFX-treated subjects, 11 (27.5%) achieved NASH resolution and improvement of ≥ 1 stage of fibrosis. Neither placebo subject met this endpoint.

Overall, 19 (47.5%) subjects achieved improvement in liver fibrosis ≥ 1 stage and no worsening of steatohepatitis, and 55.0% experienced fibrosis improvement of ≥ 1 stage.

Table 2. Summary of Histology Endpoints (Liver Biopsy Analysis Set)

Measure (Mean)	Placebo (N = 2)	EFX (once weekly dose) 28mg (N = 13)	50mg (N = 13)	70mg (N = 14)
Liver biopsy responders based on NAS system, n (%)	1 (50)	10 (76.9)	10 (76.9)	11 (78.6)
Fibrosis regression of ≥ 1 stage by NASH CRN Classification, n (%)[a]	0 (0)	7 (53.8)	10 (76.9)	5 (35.7)
Fibrosis regression of ≥ 1 stage and no worsening of steatohepatitis	0 (0.0)	6 (46.2)	8 (61.5)	5 (35.7)
Improvement in liver fibrosis ≥ 2 stages (%)[a]	0 (0)	4 (30.8)	5 (38.5)	2 (14.3)
NASH resolution and no worsening of fibrosis stage (%)[a]	1 (50.0)[b]	6 (46.2)	7 (53.8)	6 (42.3)
NASH resolution AND improvement in fibrosis stage (%)[a]	0 (0)	4 (30.8)	5 (38.5)	2 (14.3)

EFX = Efruxifermin; NAS = NAFLD Activity Score, NASH CRN = Nonalcoholic Steatohepatitis Clinical Research Network

a Liver Biopsy Evaluable Analysis Set (all subjects who had baseline and end-of-treatment liver biopsy results)

b A single placebo responder lost 25 pounds over 16 weeks (11% weight reduction)

Efruxifermin had a robust effect on the overall metabolic profile, including restoration of healthy lipoprotein profiles in subjects presenting with dyslipidemia at baseline. Significant changes at Week 16 were observed across all EFX groups in high-density lipoprotein cholesterol (HDL-C; increases ranging from 14.03 to 15.80 mg/dL), non-HDL–C (decreases ranging from 23.33 to 31.47 mg/dL), and TG (decreases ranging from 71.03 to 89.62 mg/dL). Most importantly, there were no increases in low-density lipoprotein cholesterol (LDL-C) across EFX groups. Additionally, a significant LDL-C reduction was noted in the 28-mg EFX group compared to placebo.

Clinically meaningful improvements in glycemic control and weight loss were reported at all doses. Significant reductions at Week 16 were observed in hemoglobin A1c (HbA1c) for the 50- and 70-mg EFX dose groups (–0.39% and –0.50%, respectively). Clinically meaningful improvements were also observed for insulin resistance as measured by Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), adiponectin, and C-peptide. For HOMA-IR, significant reductions in the 50- and 70-mg EFX groups were reported (7.675 and 8.775, respectively), while adiponectin levels increased significantly in a dose-related fashion by 2.8486 to 5.6437 mg/dL across all EFX groups. As expected with the improved insulin sensitivity and glycemic control, C-peptide levels were significantly lower in all EFX groups (1.724 to 2.063 µg/L). Lastly, mean weight decreases were reported in all EFX groups, with a statistically significant reduction of 2.80 kg (placebo-adjusted 3.27 kg), in the 70-mg dose group.

Two serum biomarkers of liver fibrosis were measured: the diagnostic marker Enhanced Liver Fibrosis (ELF) score, which is comprised of hyaluronic acid, tissue inhibitor of metalloproteinases 1 (TIMP-1), and amino terminal pro-peptide of type 3 procollagen (Pro-C3); and Pro-C3, a marker of new collagen synthesis or fibrogenesis.

There was a significant reduction from baseline to Week 12/Last Observation Carried Forward (LOCF) in LS mean ELF score of –0.702 ($p<0.0001$), –0.745 ($p<0.0001$), –0.435 ($p=0.0089$) in the 28-, 50-, and 70-mg EFX groups, respectively. When compared to placebo, the change from baseline in ELF score at Week 12/LOCF was statistically significant for all 3 EFX groups ($p\leq0.0341$).

Pro-C3 levels decreased from baseline after 16 weeks of treatment with EFX, indicating reduced rates of synthesis of collagen in the liver of NASH subjects. Significant reductions from baseline to Week 16/LOCF in LS mean Pro-C3 values of –6.04 ($p<0.0001$), –4.72 ($p<0.0001$), and –5.94 µg/L ($p<0.0001$) were observed in the 28-, 50-, and 70-mg EFX groups, respectively. When compared to placebo, the change from baseline in Pro-C3 at Week 16/LOCF was statistically significant for all 3 EFX groups ($p\leq0.0038$).

1.4.1.1.4. Conclusion

These improvements in the underlying driver of NASH, lipotoxicity, and in downstream pathological sequelae translated into approximately half of EFX treated subjects (with end of treatment liver biopsies) meeting the regulatory endpoints of improving fibrosis by ≥ 1 stage and no worsening of NASH, or resolving NASH and no worsening of fibrosis after 16 weeks of treatment.

Efruxifermin also ameliorated 2 common comorbidities of NASH: inadequate glycemic control and dyslipidemia. Clinically meaningful reductions in HbA1c were achieved atop existing antidiabetic medication and by enhancing insulin sensitivity, while at the same time reducing body weight. The magnitude of improvement in lipoprotein profile were sufficient to restore a normal profile; a potentially important effect given the susceptibility of NASH patients to cardiovascular disease.

Efruxifermin appeared to be generally well tolerated with an acceptable safety profile.

1.4.1.2. Cohort C

The primary objective of the study was to evaluate safety and tolerability of EFX 50 mg in subjects with compensated cirrhosis due to NASH. Secondary objectives evaluated improvement in non-invasive markers of fibrosis as well as markers of glucose and lipid metabolism. Histologic improvements were evaluated in a subset of subjects who underwent end of study biopsies.

1.4.1.2.1. Subject Disposition

Thirty subjects with biopsy-proven compensated cirrhosis (fibrosis stage 4) due to NASH were enrolled in Cohort C: 20 subjects in the EFX 50 mg group and 10 subjects in the placebo group.

Twenty-nine (96.7%) subjects completed the treatment period; 19 (95.0%) subjects in the EFX 50 mg group and 10 (100.0%) subjects in the placebo group. One subject (3.3%) in the EFX 50 mg group discontinued treatment due to an AE.

In total, 28 (93.3%) subjects completed the study: 19 (95.0%) subjects in the EFX 50 mg group and 9 (90.0%) subjects in the placebo group. Two (6.7%) subjects discontinued early from the study: 1 (5.0%) subject in the EFX 50 mg group due to an AE and 1 (10.0%) subject in the placebo group due to withdrawal of informed consent.

1.4.1.2.2. Demographics and Baseline Characteristics

The mean age of subjects in Cohort C was 59.8 years, with 36.7% of subjects being male and 63.3% of subjects being female. The majority of subjects were White (93.3%) and 43% were of Hispanic ethnicity.

As expected for this population, the mean weight and BMI at baseline were 105.0 kg and 37.1 kg/m^2, respectively. Fifteen subjects (50%) had T2D; 30% were taking a statin and approximately 17% were taking vitamin E/thiazolidinedione at the time of the Baseline visit.

Most demographic characteristics were well balanced across the 2 groups with the exception of sex (70% males in the placebo group compared to 20% in the EFX group) and mean body weight (119 kg in the placebo group vs 98 kg in the EFX group).

All 30 (100.0%) subjects had fibrosis stage 4 confirmed by local read at screening; 27 (90%) of which were subsequently confirmed to have fibrosis stage 4 at baseline by a central reader.

Baseline and end of study biopsies were evaluated by a central reader for 17 (56.7%) subjects who were confirmed (via central read) to be fibrosis stage 4 at baseline, with a mean NAS of 3.8.

As expected in subjects with fibrosis stage 4, the non-invasive markers of fibrosis were indicative of cirrhosis with an average liver stiffness value of 23.3 kPa, a mean baseline ELF score of 10.2, and the mean Pro-C3 value of 24.6 µg/L at screening. The baseline mean ALT and AST values were 32.0 and 30.5 U/L, respectively.

1.4.1.2.3. Safety

Overall, 27 (90.0%) subjects experienced a TEAE; 19 (95.0%) subjects in the EFX 50 mg group and 8 (80.0%) subjects in the placebo group. The majority of TEAEs experienced by subjects were either a Grade 1 or Grade 2 TEAE. One (3.3%) subject in the placebo group experienced a Grade 4 TEAE.

Sixteen (53.3%) subjects experienced a study drug-related TEAE, the majority of which were Grade 1 or 2. Study procedure-related TEAEs were observed in 8 (26.7%) subjects and all were Grade 1.

Adverse events of special interest (AESI) including injection site reactions and related terms, diarrhea, and decreased blood glucose, were noted in 12 (40.0%) subjects; 11 (55.0%) subjects in the EFX 50 mg group and 1 (10.0%) subject in the placebo group.

One (3.3%) subject in the placebo group experienced a SAE of pulmonary embolism, which was considered not related to study drug or study procedures. One (3.3%) subject in the EFX 50 mg group experienced multiple TEAEs leading to early discontinuation. There were no deaths in this study.

1.4.1.2.4. Efficacy

Efruxifermin-treated subjects demonstrated improvements in non-invasive markers of fibrosis including, absolute change from baseline in LS mean liver stiffness by transient elastography (–5.7 kPa for the EFX group vs –1.9 kPa for the placebo group; p=0.2226 vs placebo), Pro-C3 (–9.0 µg/L for the EFX group vs –3.4 µg/L for the placebo; p=0.0130 vs placebo), and ELF score (–0.4 for the EFX group vs 0.3 for the placebo group; p=0.0095 vs placebo).

Improvements were also noted in markers of liver injury including, absolute change from baseline in LS mean ALT (–10.3 U/L for the EFX group vs –1.3 U/L for the placebo group; p=0.0098 vs placebo) and AST (–9.6 U/L for the EFX group vs –4.5 U/L for the placebo group; p=0.0752 vs placebo).

As observed in the main study, significant improvements were also seen in lipoprotein profiles, including change from baseline in LS mean LDL-C (–12.1 mg/dL for the EFX group vs 9.7 mg/dL for the placebo group; p=0.0012 vs placebo), HDL-C (14.9 mg/dL for the EFX group vs 1.4 mg/dL for the placebo group; p=0.0125 vs placebo), non-HDL—C (–20.4 mg/dL for the EFX group vs 9.3 mg/dL for the placebo group; p=0.0001 vs placebo), and TG (–39.7 mg/dL for the EFX group vs –4.6 mg/dL in the placebo group; p=0.0089 vs placebo).

In addition, markers of insulin sensitivity and glycemic control significantly improved, including change from baseline in LS mean HbA1c (–0.4% for the EFX group vs 0.1% for the placebo group; p=0.0011 vs placebo), C-Peptide (–1.1 ug/L in the EFX group vs –0.5 ug/L for the placebo group; p=0.3634 vs placebo), insulin (–7.3 mIU/L for the EFX group vs 2.4 mIU/L for the placebo group; p=0.1664 vs placebo), HOMA-IR (–2.7 for the EFX group vs 0.7 for the placebo group; p=0.1495 vs placebo), and adiponectin (5.6 mg/dL for the EFX group vs 0.6 mg/dL for the placebo group; p=0.0027).

Although the study was originally designed to evaluate non-invasive markers of fibrosis as secondary endpoints, the protocol was subsequently amended to allow voluntary biopsies following 16 weeks of treatment. Paired biopsies were available in 17 subjects (n = 12 on EFX and n = 5 on placebo). Among the 12 EFX-treated subjects with paired biopsies, 4 (33%) subjects achieved fibrosis improvement without worsening of NASH and 3 (25%) subjects achieved NASH resolution, compared to 0 of 5 patients receiving placebo. Seven (58%) subjects in the EFX group achieved ≥ 2-point improvement in NAS compared with 1 (20%) subject receiving placebo. In total, 9 (75%) of the EFX-treated subjects demonstrated some histological improvement as measured by fibrosis improvement, NASH resolution, or NAS improvement ≥ 2 points, compared with 1 (20%) subject in the placebo group.

1.4.1.2.5. Conclusion

Results of Cohort C confirmed the findings from the Main Study with respect to histologic improvements in fibrosis and NASH, improvements in non-invasive markers of fibrosis, as well as markers of liver injury and glucose and lipid metabolism.

This reproducibility of improvements in fibrosis and markers of fibrosis in 2 independent cohorts further affirms the anti-fibrotic effects of EFX. Efruxifermin 50 mg was well tolerated with a similar safety profile as that observed in subjects with fibrosis stages 1—3.

These observations, combined with the high unmet medical need to identify safe and effective therapies in subjects with cirrhosis due to NASH, warrant further evaluation of EFX in this population.

1.5. Pharmacokinetic Results

Early clinical pharmacology studies included single and multiple ascending dose studies in T2D subjects. More recently, a pharmacokinetic (PK) model was developed based on 16 weeks of QW dosing in NASH subjects with F1-F3 fibrosis and compensated cirrhosis (F4, Child Pugh A) in the Phase 2a BALANCED study (AK-US-001-0101) and on 24 weeks of QW dosing in NASH subjects with F2-F3 fibrosis in the Phase 2b HARMONY Study (AK-US-001-0102).

Once weekly dosing is supported by this population PK model which estimates that the half-life ($t_{1/2}$) is approximately 2.5-3.5 days, with time to maximum concentration (T_{max}) approximately 1.7-1.9 days post-dose. After 16 weeks of dosing in the BALANCED study, serum PK profiles indicate linear systemic exposure across the dose range 28 to 70 mg EFX, with steady state levels achieved after approximately 4 weeks of dosing. Pharmacokinetic results following 24

weeks of QW dosing in HARMONY indicate dose-responsive, generally linear PK based on steady state trough levels. Clearance of EFX in the model appears to be controlled by flip-flop kinetics where the observed half-life is determined by rate of absorption.

Based on a population PK model of data from the Phase 2a (BALANCED) and Phase 2b (HARMONY), studies, body weight was a significant covariate with standard allometric scaling exponents on clearance and central volume of distribution. Dose was also a significant covariate on clearance. Age, sex, height, and anti-drug antibody (ADA) status did not appear to influence PK. No clear trend was observed by baseline fibrosis score (F1 to F4) in the population PK model.

1.6. Rationale for this Study

Non-alcoholic fatty liver disease is a global health burden; almost 25% of the global population is affected by NAFLD and/or its complications. It is the most common chronic and progressive liver disease, especially in industrialized countries (Younossi et al. 2016). It is forecasted that by 2030 there will be more than 8 million patients with late-stage NASH in the US (Estes et al. 2018). The health burden of NASH extends beyond liver-related outcomes, i.e., cirrhosis, HCC, liver failure, and liver-related mortality (Dulai et al. 2017, Ekstedt et al. 2015, Hagström et al. 2017) to greatly increased risk of cardiovascular disease and diabetic complications (Younossi et al. 2016). In pre-cirrhotic NASH, cardiovascular disease is the leading cause of morbidity and mortality (Chalasani et al. 2018). Fibrosis of the liver is the critical factor driving the progression from NASH to end-stage liver disease and patients with cirrhosis are at the highest risk of progression (Angulo et al. 2015).

Efruxifermin is a potent and long-acting FGF21 analog highly expressed in tissues relevant to metabolic function, including the liver, adipose tissue, and pancreas (Fon Tacer et al. 2010, Nishimura et al. 2000). Efruxifermin has been engineered to increase human FGF21's $t_{1/2}$ (up to 3.5 days) sufficiently to enable QW dosing, while retaining the native biological activity of FGF21 (Kaufman et al. 2020). These unique properties of EFX enable its potential to address the core processes underlying NASH pathogenesis: improving energy metabolism in the liver, reducing hepatocyte stress, mitigating inflammation, and resolving fibrosis (Lee et al. 2016, Tanaka et al. 2015, Xu et al. 2009).

Data from the Main Study of the Phase 2a study AK-US-001-0101 demonstrate that EFX administered in doses between 28 and 70 mg SC QW for 16 weeks significantly improved health of the liver with substantial reductions of fat and in markers of liver injury. These were associated with encouraging histologic improvements including resolution of steatohepatitis and crucial improvement of fibrosis after just 16 weeks of treatment. In addition, EFX improved whole body metabolic status with better glycemic control and amelioration of dyslipidemia, combined with a clear trend to weight loss. Similar results were observed in subjects with NASH and stage 4 fibrosis or compensated cirrhosis following 16 weeks of treatment with EFX 50 mg, demonstrating improvements in fibrosis on histology, improvements in markers of liver injury and noninvasive markers of fibrosis, as well as robust improvements in lipoprotein profile and glucose metabolism. These changes in 2 independent cohorts spanning the spectrum of NASH disease severity including compensated cirrhosis establish a foundation for sustained

improvement in liver health and further resolution of liver injury and fibrosis beyond that observed after 16 weeks of treatment.

The **main study** is a Phase 2b, randomized, double-blind, placebo-controlled study evaluating the safety and efficacy of EFX in subjects with compensated cirrhosis due to NASH. Subjects will be fibrosis stage 4 and dosed weekly with EFX 50 mg, EFX 28 mg, or placebo. Following the 36-week analysis, selection of the 28-mg or 50-mg EFX dose will be based on achieving the efficacy endpoints and in consideration of the safety and exploratory analysis. Subjects will be followed for long-term safety until Week 96.

The dose and length of administration of the drug in the **main study** AK-US-001-0103 are supported by safety data from nonclinical studies and from the safety and efficacy data from the Phase 2a study.

Nonalcoholic steatohepatitis with fibrosis stage 4 or cirrhosis is a condition with a high risk of progression to end-stage liver disease including an increased risk of HCC. Thus, reversing the fibrotic process in addition to ameliorating the metabolic dysfunction that drives NASH pathogenesis is paramount to improving the prognosis of this condition. The current gold standard for assessing fibrosis is liver biopsy. As such, improvement in liver fibrosis without worsening of NASH as assessed by liver biopsy will be evaluated as the primary efficacy parameter in the **main study** portion of this Phase 2b study. Efficacy will be determined by changes in liver histology. In addition, the effect of EFX on clinical outcome events will be evaluated based on adjudicated hepatic clinical event rates that include hepatic decompensation, liver transplantation, and all-cause mortality. Due to the increased risk of cardiovascular morbidity and mortality in NASH (Angulo et al. 2015, Ekstedt et al. 2015, Targher et al. 2016), the impact of EFX on metabolic parameters (e.g., lipids and insulin resistance) will be monitored and cardiovascular events will be prospectively recorded and adjudicated. Liver biopsy has numerous limitations including its invasiveness and the potential for serious complications, which deter many patients from seeking evaluation for NAFLD or enrolling in clinical trials of novel therapies. Liver biopsy is also limited by cost, sampling error, and variability in histopathological interpretation. Due to these limitations, the development and validation of non-invasive markers of liver injury has emerged as a clinical and research priority. To further the development of such tools, the **main study** will also incorporate non-invasive endpoints for the assessment of fibrosis including serum markers (e.g., ELF™ test and Pro-C3) and imaging (e.g., liver stiffness by FibroScan® and magnetic resonance elastography [MRE]). [redacted]

Cohort D is a randomized, double-blind, placebo-controlled cohort evaluating the safety and tolerability of EFX compared to placebo when added to an existing glucagon-like-peptide-1 (GLP-1) receptor agonist in subjects with T2D and fibrosis (stages 1—3) due to NASH. Subjects will be dosed weekly with EFX 50 mg or placebo for up to 12 weeks.

Patients with fibrotic non-alcoholic steatohepatitis typically have multiple metabolic comorbidities (e.g., obesity, T2D, insulin resistance, and dyslipidemia). Presence of T2D has been identified as a risk factor for rapid progression of liver disease, including progression of fibrosis. Given the potential for more rapid disease progression, patients with T2D and liver fibrosis due to NASH represent a high-risk population warranting a therapeutic approach that can ameliorate metabolic abnormalities in addition to improving liver health. Concomitant treatment of EFX and GLP-1 may offer synergistic benefit of better metabolic control and NASH fibrosis improvement, which is the rationale for targeting the patient population in **Cohort D**.

The safety and tolerability of concomitant EFX and GLP-1 receptor agonist use will be assessed through the reporting of AEs, clinical laboratory tests, ECGs, ultrasounds, vital sign assessments, and concomitant medication usage. To further the development and validation of non-invasive markers of liver fibrosis and liver fat content, **Cohort D** will also incorporate non-invasive endpoints including imaging (e.g., liver stiffness by FibroScan® and MRI-PDFF) and serum markers (e.g., ELF™ test and Pro-C3).

1.7. Rationale for Selection of Dose

The doses of EFX chosen for evaluation in this study, 28 mg and 50 mg QW, are supported by a combination of safety and efficacy data from Phase 1 and 2 studies in the clinical development program.

As described in Section 1.4, data from the main portion of the Phase 2a study AK-US-001-0101 demonstrate that EFX administered in doses from 28 to 70 mg SC QW for 16 weeks significantly improved health of the liver with substantial reductions of fat and in markers of liver injury. These data were associated with encouraging histologic improvements with EFX including improvement in fibrosis by ≥ 1 stage and by ≥ 2 stages after just 16 weeks of treatment, attesting to the anti-fibrotic effects of EFX. Treatment with EFX was also associated with resolution of steatohepatitis. In addition, EFX improved whole body metabolic status with better glycemic control and amelioration of dyslipidemia, combined with a clear trend to weight loss. These changes establish a foundation for sustained improvement in liver health and further resolution of liver injury and fibrosis beyond that observed after 16 weeks treatment. The magnitude and rapidity of reduction in liver fat after only 12 weeks treatment were substantial, such that levels of liver fat are normalized ($\leq 5\%$) in a substantial proportion of treated patients.

The 50-mg EFX dose when compared with the 70-mg EFX dose was associated with near-maximal effects. The 28-mg EFX dose showed encouraging effects on liver health and dyslipidemia but given the short treatment duration of 16 weeks its impact on glycemic control, insulin sensitivity, and body weight appeared less.

The 28-mg and 50-mg EFX doses were well tolerated, with fewer subjects experiencing study drug related TEAE or discontinuing from the study due to AEs than the 70-mg EFX dose. A total of 7 subjects discontinued from study drug due to TEAEs (4 in the 70-mg EFX group, 0 in the 50-mg EFX group, 2 in the 28-mg EFX group, and 1 on placebo). The 50-mg EFX dose was also evaluated in a separate cohort, Cohort C, in subjects with stage 4 fibrosis or compensated cirrhosis, and demonstrated comparable safety, tolerability, and efficacy to that observed in the

main study portion of AK-US-001-0101. Fibrosis improvement by at least 1 stage with no worsening of NASH was observed in 33% of subjects with fibrosis stage 4 treated with EFX 50 mg, with a tolerability profile that was consistent with that seen in earlier stage NASH subjects (fibrosis stages 1—3).

Additionally, nonclinical toxicology studies up to 26 weeks in duration have been conducted in monkeys and rats, at exposure margins multiple folds above the expected clinical exposure. Efruxifermin exposures in non-cirrhotic subjects at the respective NOAELs in rats and monkeys are expected to range from approximately 8- to 27-fold based on C_{max} and approximately 9- to 41-fold based on AUC_{0-t}.

In AK-US-001-0101, a significant reduction in HbA1c was observed with EFX 50 mg, and the magnitude of reduction was more pronounced in subjects with T2D. As a result of the positive effect of the 50 mg dose on glycemic parameters in subjects with T2D, **Cohort D** will evaluate EFX 50 mg in subjects with T2D and liver fibrosis stages 1—3. EFX 50 mg appeared to be safe and well-tolerated in subjects with T2D, including the subgroup of T2D subjects taking concomitant GLP-1 receptor agonists (n=5), as well as the overall population of the main study and Cohort C (AK-US-001-0101).

Taking all data into consideration, the **main study** will evaluate the 28 and 50 mg doses of EFX in subjects with compensated cirrhosis due to NASH, combining the likely effective dose range with acceptable safety and tolerability. **Cohort D** will evaluate the 50 mg dose of EFX in subjects with T2D and liver fibrosis (stages 1—3) due to NASH, noting the clinically meaningful reduction of HbA1c observed in AK-US-001-0101 in subjects with T2D.

1.8. Compliance

This study will be conducted in compliance with this protocol, Good Clinical Practice (GCP), and all applicable regulatory requirements.

2. STUDY OBJECTIVES AND ENDPOINTS

Table 3. Main Study Objectives and Endpoints

Primary Objective	Primary Endpoint
• To evaluate the effect of EFX compared to placebo on fibrosis regression in subjects with compensated cirrhosis due to NASH at Week 36	• Proportion of subjects who achieve ≥ 1 stage improvement in fibrosis (based on NASH CRN fibrosis score) and no worsening of steatohepatitis at Week 36
Secondary Objectives	**Secondary Endpoints**
• To evaluate the effect of EFX compared to placebo on achieving NASH resolution in subjects with compensated cirrhosis due to NASH at Week 36 and Week 96	• Proportion of subjects who achieve NASH resolution (defined as a NAS of 0—1 for inflammation and 0 for ballooning) as determined by the NASH CRN criteria at Week 36 and Week 96
• To evaluate the effect of EFX compared to placebo on achieving NASH resolution AND improvement in fibrosis at Week 36 and Week 96	• Proportion of subjects who achieve NASH resolution (defined as a NAS of 0—1 for inflammation and 0 for ballooning) and ≥ 1 stage improvement in fibrosis (based on NASH CRN fibrosis score) at Week 36 and Week 96
• To evaluate the effect of EFX compared to placebo on fibrosis regression in subjects with compensated cirrhosis due to NASH at Week 96	• Proportion of subjects who achieve ≥ 1 stage improvement in fibrosis (based on NASH CRN fibrosis score) and no worsening of steatohepatitis at Week 96
• To evaluate the effect of EFX compared to placebo on fibrosis regression in subjects with compensated cirrhosis due to NASH at Week 36 and Week 96	• Proportion of subjects who achieve ≥ 1 stage improvement in fibrosis (based on NASH CRN fibrosis score) at Week 36 and Week 96
• To evaluate the effect of EFX compared to placebo on non-invasive markers of fibrosis	• Change from baseline in ELF score, three components of ELF (including PIIINP, HA, and TIMP-1), Pro-C3, liver stiffness assessed by FibroScan®, and FibroScan®-AST (FAST) score
• To evaluate the effect of EFX compared to placebo on lipoproteins	• Change from baseline in TG, total cholesterol, HDL-C, non-HDL—C, and LDL-C
• To evaluate the effect of EFX compared to placebo on markers of insulin sensitivity and glycemic control	• Change from baseline in HbA1c, C-peptide, adiponectin, insulin, and HOMA-IR
• To evaluate the effect of EFX compared to placebo on weight change	• Change from baseline in body weight
• To assess the immunogenicity of EFX	• Detect and measure ADA, including NAb, against EFX
• To assess the safety and tolerability of EFX	• Safety and tolerability will be assessed through the reporting extent of exposure, AEs, clinical laboratory tests, ECGs, ultrasounds, vital sign assessments, and concomitant medication usage



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• [redacted]	• [redacted]
• [redacted]	• [redacted] • [redacted]

ADA = anti-drug antibody; AE: adverse event; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMD = bone mineral density; BSAP = bone-specific alkaline phosphatase; C3M = type III collagen; CAP = controlled attenuation parameter; CLDQ-NASH = Chronic Liver Disease Questionnaire-Nonalcoholic Steatohepatitis; CRN = Clinical Research Network ; CTX-1 = C-telopeptide of type I collagen; DXA = dual-energy X-ray absorptiometry; ECG = electrocardiogram; eGFR = estimated glomerular filtration rate; EFS = event-free survival; EFX = Efruxifermin; ELF = Enhanced Liver Fibrosis; GGT = gamma glutamyl transferase; HA = hyaluronic acid; HbA1c = hemoglobin Alc; HDL-C = high-density lipoprotein cholesterol; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; hs-CRP = high-sensitivity C-reactive protein; IGF-1 = insulin-like growth factor 1; INR = International Normalized Ratio; LDL-C = low-density lipoprotein cholesterol; MELD = Model for End-Stage Liver Disease; MRE = magnetic resonance elastography; NAb = neutralizing antibody; NAS = NAFLD Activity Score ; NASH = nonalcoholic steatohepatitis; P1NP = procollagen type 1 N pro-peptide; PIIINP = amino-terminal pro-peptide of type 3 procollagen; Pro-C3 = pro-peptide of type 3 procollagen; PTH = parathyroid hormone; T2D = type 2 diabetes; TG = triglycerides; TIMP-1 = tissue inhibitor of metalloproteinase-1; TSH = thyroid stimulating hormone; VLDL-C = very low-density lipoprotein cholesterol; WPAI-NASH = Work Productivity and Activity Impairment for Nonalcoholic Steatohepatitis

Table 4. Cohort D Study Objectives and Endpoints

Primary Objective	Primary Endpoint
• To evaluate the safety and tolerability of EFX compared to placebo when added to an existing GLP-1 receptor agonist in subjects with T2D and liver fibrosis due to NASH	• Safety and tolerability will be assessed through the reporting extent of exposure, AEs, clinical laboratory tests, Child-Pugh score, ECGs, vital sign assessments, and concomitant medication usage
Secondary Objectives	**Secondary Endpoints**
• To evaluate the effect of EFX compared to placebo when added to an existing GLP-1 receptor agonist on liver fat content	• Change from baseline in liver fat content as assessed by MRI-PDFF at Week 12 • Proportion of subjects who achieve a relative reduction of at least 30% in liver fat content as measured by MRI-PDFF at Week 12 • Proportion of subjects who achieve a relative reduction of at least 50% in liver fat content as measured by MRI-PDFF at Week 12 • Proportion of subjects who achieve a relative reduction of at least 70% in liver fat content as measured by MRI-PDFF at Week 12 • Proportion of subjects who achieve normalization of liver fat (liver fat content ≤ 5%) at Week 12
• To evaluate the effect of EFX compared to placebo when added to an existing GLP-1 receptor agonist on markers of liver injury and function, and hemostasis	• Change from baseline in ALT, AST, ALP, GGT, total bilirubin, direct bilirubin, INR, PAI-1, fibrinogen, albumin, platelets, uric acid, and MELD score
• To evaluate the effect of EFX compared to placebo when added to an existing GLP-1 receptor agonist on non-invasive markers of fibrosis	• Change from baseline in ELF score, three components of ELF (including PIIINP, HA, and TIMP-1), Pro-C3, liver stiffness assessed by FibroScan®, and FAST score
• To evaluate the effect of EFX compared to placebo when added to an existing GLP-1 receptor agonist on lipoproteins	• Change from baseline in TG, total cholesterol, HDL-C, non-HDL—C, LDL-C, apolipoprotein B, apolipoprotein C3, and lipoprotein(a)
• To evaluate the effect of EFX compared to placebo when added to an existing GLP-1 receptor agonist on markers of insulin sensitivity and glycemic control	• Change from baseline in HbA1c, C-peptide, adiponectin, insulin, and HOMA-IR • Proportion of subjects who achieve normalization of HbA1c (defined as ≥ 6.5% at baseline and < 6.5% at Week 12)
• To evaluate the effect of EFX compared to placebo when added to an existing GLP-1 receptor agonist on weight change	• Change from baseline in body weight
• To assess the immunogenicity of EFX	• Detect and measure ADA, including NAb, against EFX
[redacted]	[redacted]

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• [redacted]	• [redacted]

ADA = anti-drug antibody; AE: adverse event; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BSAP = bone-specific alkaline phosphatase; C3M = type III collagen; CAP = controlled attenuation parameter; CTX-1 = C-telopeptide of type I collagen; ECG = electrocardiogram; eGFR = estimated glomerular filtration rate; EFX = Efruxifermin; ELF = Enhanced Liver Fibrosis; FAST = FibroScan®-AST; GLP-1 = glucagon-like-peptide-1; GGT = gamma glutamyl transferase; HA = hyaluronic acid; HbA1c = hemoglobin Alc; HDL-C = high-density lipoprotein cholesterol; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; hs-CRP = high-sensitivity C-reactive protein; INR = International Normalized Ratio; LDL-C = low-density lipoprotein cholesterol; MELD = Model for End-Stage Liver Disease; MRI-PDFF = Magnetic Resonance Imaging-Proton Density Fat Fraction; NAb = neutralizing antibody; NASH = nonalcoholic steatohepatitis; P1NP = procollagen type 1 N pro-peptide; PIIINP = amino-terminal pro-peptide of type 3 procollagen; PAI-1 = plasminogen activator inhibitor-1; Pro-C3 = pro-peptide of type 3 procollagen; PTH = parathyroid hormone; T2D = type 2 diabetes; TG = triglycerides; TIMP-1 = tissue inhibitor of metalloproteinase-1; TSH = thyroid-stimulating hormone

3. INVESTIGATIONAL PLAN

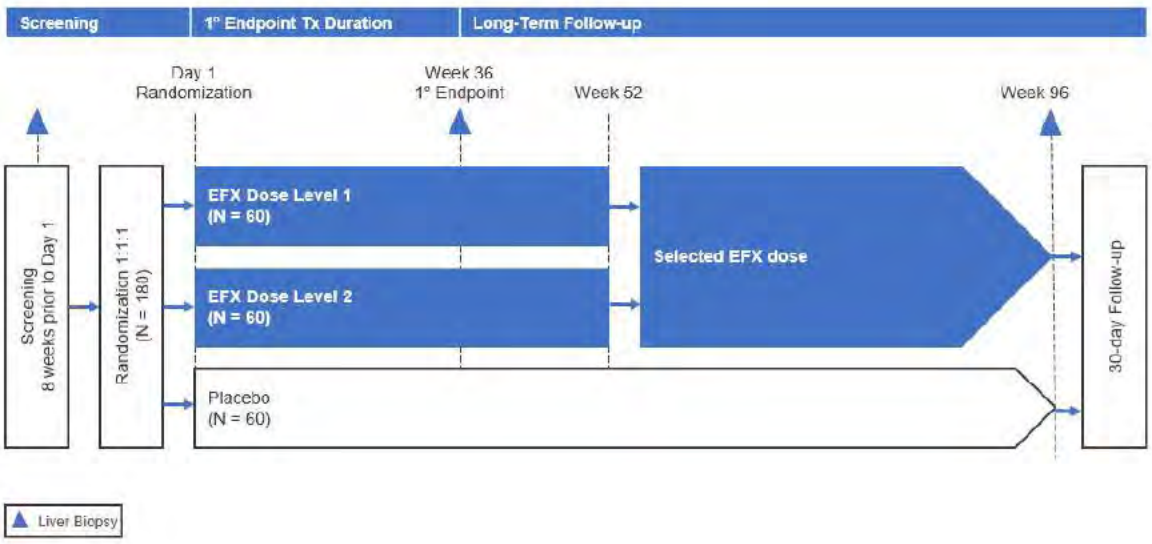
3.1. Overall Study Design and Plan

The **main study** is a Phase 2b, randomized, double-blind, placebo-controlled study evaluating the safety and efficacy of EFX in subjects with compensated cirrhosis due to NASH. Subjects will have fibrosis stage 4 without any history of decompensation and will be dosed QW with EFX 50 mg, EFX 28 mg, or placebo for up to 96 weeks.

After all subjects have completed the Primary Endpoint Treatment Duration (i.e., completed 36 weeks of treatment or permanently discontinued the study prior to the Week 36 visit), the primary endpoint analysis will be performed to select a single dose of EFX (50 mg or 28 mg) for further evaluation. The Sponsor will seek alignment from the applicable regulatory authorities on the dose selected prior to clinical use. If a subject refuses to complete the Week 36 liver biopsy, the subject will be discontinued from the study at Week 36. Only a 30-day Follow-up visit is required following discontinuation of study drug.

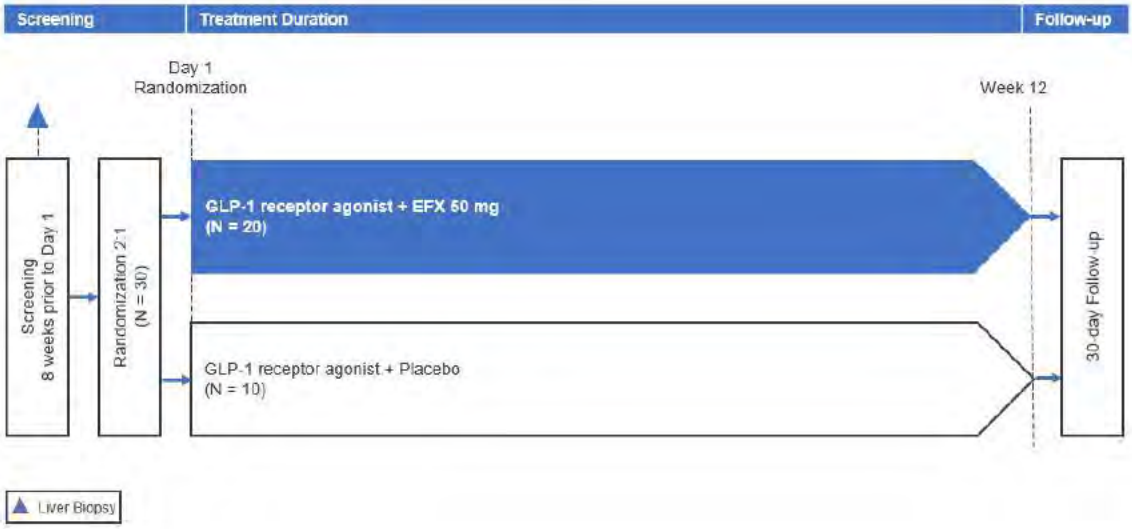
Subjects receiving EFX 50 mg or EFX 28 mg may be eligible to receive the selected dose of EFX. Roll over to the selected dose of EFX will occur only after all active subjects have completed 52 weeks of treatment and the selected dose is confirmed. Subjects assigned to the placebo group will continue to receive placebo but may be offered open-label EFX in a separate roll over study after completing 96 weeks of treatment.

Figure 3-1. Study Design – Main Study



Cohort D is a randomized, double-blind, placebo-controlled cohort evaluating the safety and tolerability of EFX compared to placebo when added to an existing GLP-1 receptor agonist in subjects with T2D and liver fibrosis (stages 1—3) due to NASH. Subjects will be dosed QW with EFX 50 mg or placebo for up to 12 weeks.

Figure 3-2. Study Design – Cohort D



3.2. Randomization and Treatment Assignment

Main Study

On Day 1, eligible subjects will be randomized in a blinded fashion in a 1:1:1 ratio to 1 of 3 treatment groups. Randomization will be stratified by T2D status (Yes vs No) and diagnosis of cryptogenic cirrhosis presumed secondary to NASH (Yes vs No) at baseline.

Treatment Group	Study Drug Assignment
EFX 50 mg (N = 60)	EFX 50 mg, subcutaneous injection, once weekly
EFX 28 mg (N = 60)	EFX 28 mg, subcutaneous injection, once weekly
Placebo (N = 60)	Placebo, subcutaneous injection, once weekly

EFX = Efruxifermin

Cohort D

On Day 1, eligible subjects will be randomized in a blinded fashion in a 2:1 ratio to 1 of 2 treatment groups as shown below. Randomization will be stratified by liver fibrosis stage (1 vs 2-3) at baseline.

Treatment Group	Study Drug Assignment
EFX 50 mg (N = 20)	EFX 50 mg, subcutaneous injection, once weekly
Placebo (N = 10)	Placebo, subcutaneous injection, once weekly

EFX = Efruxifermin

3.3. Study Duration and Number of Centers

Main Study: Study participation may last up to 108 weeks, including an 8-week screening period, 96-week treatment period, and a 30-day Follow-up visit.

Cohort D: Study participation may last up to 24 weeks, including an 8-week screening period, 12-week treatment period, and a 30-day Follow-up visit.

This study will be conducted at approximately 60 centers across North America. Additional sites may be added, depending on subject accrual rates.

3.4. Early Termination

Subjects who have received at least 1 dose of study drug and discontinue the study prematurely will return to the clinic within 5 days of discontinuing study drug to complete the Early Termination (ET) visit.

Subjects with ongoing clinically significant clinical or laboratory findings will be followed until the finding is resolved or medically stable; reasonable attempts will be made to follow up with subjects. The subject's participation will end once all study assessments and the 30-day Follow-up visit have been completed.

The ET visit assessments are outlined in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C).

3.5. 30-day Follow-up

The 30-day Follow-up visit will be completed 30 days following the last dose of study drug.

The 30-day Follow-up visit assessments are outlined in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C).

3.6. Post Study Care

After the subject has completed his/her study participation, the long-term care of the participant will remain the responsibility of their primary treating physicians.

Subjects assigned to the placebo group of the **main study** may be offered open-label EFX in separate roll over study after completing the Long-Term Follow-up.

4. STUDY POPULATION SELECTION

4.1. Study Population

The **main study** will randomize approximately 180 subjects with biopsy-proven compensated cirrhosis (fibrosis stage 4) due to NASH, and no evidence or history of hepatic decompensation.

Cohort D will randomize approximately 30 subjects with biopsy-proven fibrosis (stages 1—3) due to NASH and T2D who are being treated with a stable dose of a GLP-1 receptor agonist.

4.2. Inclusion Criteria

4.2.1. Main Study

Subjects in the **main study** must meet all of the following inclusion criteria to be eligible for participation in this study:

1. Males and non-pregnant, non-lactating females between 18 and 75 years of age, inclusive, on the day of signing informed consent

2. Previous history or presence of T2D or 2 out of 4 components of metabolic syndrome (obesity, dyslipidemia, elevated blood pressure, elevated fasting glucose)

3. Subjects who do not have a historical liver biopsy specimen, which is defined as a liver biopsy specimen collected ≤ 365 days prior to screening with prior confirmation of fibrosis stage 4 that is available for submission to a central reader for eligibility assessment, must meet either inclusion criterion 3a **OR** 3b prior to collection of a liver biopsy specimen during the Screening visit:

   a. Liver stiffness measurement (LSM) ≥ 15 kPa

      Note: All subjects must complete a FibroScan® examination during the screening period, unless historical values for FibroScan® performed within 90 days prior to the Screening visit are available

   b. ELF score > 9.8

      Note: Subjects who do not have a historical liver biopsy specimen require a screening ELF score. Historical results for an ELF test performed within 90 days prior to the Screening visit may be accepted as the screening value

4. Central laboratory tests at screening that meet all of the following criteria:

   a. Albumin ≥ 3.5 g/dL

   b. Estimated glomerular filtration rate (eGFR) ≥ 45 mL/min, as calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation

c. HbA1c ≤ 9.5%

d. INR ≤ 1.3, unless due to therapeutic anticoagulation

e. Direct bilirubin ≤ 0.3 mg/dL

f. Total bilirubin ≤ 1.3 mg/dL, unless due to an alternate etiology such as Gilbert's syndrome or hemolytic anemia

g. Creatine kinase (CK) < 3 × upper limit of normal (ULN)

h. Platelet count ≥ 100,000/µL

i. TG level ≤ 500 mg/dL

j. ALT ≤ 5 × ULN

k. AST ≤ 5 × ULN

l. ALP < 1.5 × ULN

5. Documented historical stability of ALT and AST levels, as evidenced by no significant worsening of ALT and AST values at screening relative to historical values obtained 28 days to 180 days prior to the Screening visit and based on the following parameters:

   a. If the historical and screening ALT and AST values are both ≤ 1.5 × ULN, there is no limit to the difference between the values

   b. If at least 1 of the historical or screening ALT or AST values is > 1.5 × ULN and shows worsening at screening, the percent increase must be ≤ 50%

   Note: Subjects without historical ALT and AST evaluations must have ALT and AST repeated during the screening period (Pre-baseline visit) at minimum 28 days between blood draws to confirm criterion 5a or 5b above

6. Biopsy-proven compensated cirrhosis (fibrosis stage 4) due to definitive NASH or cryptogenic cirrhosis presumed secondary to NASH as defined in Appendix G. Must have had a liver biopsy specimen collected during screening or ≤ 365 days prior to screening with confirmation of fibrosis stage 4 by a central pathologist. Subjects with cryptogenic cirrhosis presumed secondary to NASH will be limited to approximately 20% of the total study population

7. Subjects on Vitamin E ≥ 800 IU/day, antidiabetic, weight loss, or lipid-modifying medication(s) must be on a stable dose (defined as no significant change in prescription efficacy, initiation of medication, or medication discontinuation) as outlined in Table 6. A switch from one drug to another in the same class should be discussed with the Medical Monitor to confirm subject eligibility

8. Willing and able to give written informed consent prior to any study specific procedures being performed

9. Female subjects of childbearing potential (see definition in Appendix D) must have a negative serum pregnancy test at screening and a negative urine pregnancy test at baseline/Day 1

10. Male and female subjects of childbearing potential who engage in heterosexual intercourse must agree to use protocol specified method(s) of contraception as described in Appendix D.

4.2.2. Cohort D

Subjects in **Cohort D** must meet all of the following inclusion criteria to be eligible for participation in this study:

1. Males and non-pregnant, non-lactating females between 18 and 75 years of age, inclusive, on the day of signing informed consent

2. HbA1c ≤ 9.5% at screening

3. Diagnosis of T2D and on a stable dose of a GLP-1 receptor agonist for at least 90 days prior to screening as outlined in Table 7

4. Subjects who do not have a historical liver biopsy specimen, which is defined as a liver biopsy specimen collected ≤ 365 days prior to screening with prior confirmation of fibrosis stage 1, 2, or 3 from a pathologist, must meet inclusion criterion 4a **AND** 4b prior to collection of a liver biopsy specimen during the Screening visit:

   a. LSM ≥ 7.0 kPa

   Note: All subjects must complete a FibroScan® examination during the screening period, unless historical values for FibroScan® performed within 90 days prior to the Screening visit are available

   b. AST > 17 U/L for females and > 20 U/L for males

5. Central laboratory tests at screening that meet all of the following criteria:

   a. eGFR ≥ 45 mL/min, as calculated by the CKD-EPI equation

   b. INR ≤ 1.3, unless due to therapeutic anticoagulation

   c. Direct bilirubin ≤ 0.3 mg/dL

   d. Total bilirubin ≤ 1.3 mg/dL, unless due to an alternate etiology such as Gilbert's syndrome or hemolytic anemia

   e. CK < 3 × ULN

f. Platelet count ≥ 140,000/μL

g. TG level ≤ 500 mg/dL

h. ALT ≤ 5 × ULN

i. AST ≤ 5 × ULN

j. ALP < 2 × ULN

6. Documented historical stability of ALT and AST levels, as evidenced by no significant worsening of ALT and AST values at screening relative to historical values obtained 28 days to 180 days prior to the Screening visit and based on the following parameters:

   a. If the historical and screening ALT and AST values are both ≤ 1.5 × ULN, there is no limit to the difference between the values

   b. If at least 1 of the historical or screening ALT or AST values is > 1.5 × ULN and shows worsening at screening, the percent increase must be ≤ 50%

   Note: Subjects without historical ALT and AST evaluations must have ALT and AST repeated during the screening period (Pre-baseline visit) at minimum 28 days between blood draws to confirm criterion 6a or 6b above

7. Biopsy-proven liver fibrosis due to NASH. Must have had a liver biopsy specimen collected during screening or ≤ 365 days prior to screening with confirmation of fibrosis stage 1, 2, or 3 from a pathologist

8. Subjects on Vitamin E ≥ 400 IU/day, additional antidiabetic medications (such as SGLT2 inhibitors), weight loss, or lipid-modifying medication(s) must be on a stable dose (defined as no significant change in prescription efficacy, initiation of medication, or medication discontinuation) as outlined in Table 7. A switch from one drug to another in the same class should be discussed with the Medical Monitor to confirm subject eligibility

9. Willing and able to give written informed consent prior to any study specific procedures being performed

10. Female subjects of childbearing potential (see definition in Appendix D) must have a negative serum pregnancy test at screening and a negative urine pregnancy test at baseline/Day 1

11. Male and female subjects of childbearing potential who engage in heterosexual intercourse must agree to use protocol specified method(s) of contraception as described in Appendix D

4.3. Exclusion Criteria

4.3.1. Main Study

Subjects in the **main study** who meet *any* of the following exclusion criteria are not to be enrolled in this study:

1. Weight loss > 10% in the 90 days prior to screening until randomization or from the time of collection of the liver biopsy specimen used to assess subject eligibility until randomization, whichever is longer

2. Type 1 diabetes

3. Unstable T2D defined as:

   a. Insulin dose adjustment > 35% within 30 days prior to screening

   b. Any prior history of diabetic ketoacidosis and/or hyperglycemic hyperosmolar state

4. Hypoglycemia unawareness, hospitalization due to hypoglycemia, or history of severe hypoglycemia (hypoglycemia requiring outside assistance to regain normal neurologic status) within 90 days prior to screening

5. Subjects with osteoporosis, defined as a T-score of ≤ –2.5 at the femoral neck, total hip, or lumbar spine based on a centrally read dual-energy X-ray absorptiometry (DXA) scan performed during screening

   Note: A historical DXA scan performed within 90 days prior to screening may be accepted as the screening DXA scan. The historical scan must have been performed on a scanner previously qualified by the central imaging vendor that is available for use at post-baseline visits

6. Poorly controlled hypertension (systolic blood pressure > 160 mm Hg, or diastolic blood pressure > 100 mm Hg) at screening

   Note: Vital signs for eligibility assessment may be repeated one time at the Investigator's discretion

7. Current or prior history of decompensated liver disease requiring medical management, including ascites, hepatic encephalopathy (HE), or variceal bleeding

8. MELD score > 12, unless due to therapeutic anti-coagulation

9. Child-Pugh score > 6 (Class B or C), unless due to therapeutic anti-coagulation

10. History of pancreatitis

Note: Subjects with a history of gallstone pancreatitis and subsequent cholecystectomy may be allowed to participate with prior written approval from the Medical Monitor

11. Chronic hepatitis B virus (HBV) infection (hepatitis B surface antigen [HBsAg] positive) or acute hepatitis A infection (hepatitis A immunoglobulin M [IgM] antibody positive). For subjects with positive hepatitis B core antibody (HBcAb), HBV DNA by quantitative polymerase chain reaction (PCR) will be required

12. Chronic hepatitis C virus (HCV) infection (HCV antibody [Ab] and HCV RNA positive). Subjects cured of HCV infection < 2 years prior to the Screening visit (based on date of RNA PCR negative confirmation following conclusion of treatment) are not eligible. Asymptomatic subjects with spontaneous resolution of HCV infection (i.e., without RNA PCR negative confirmation following conclusion of treatment) may be allowed to participate with prior written approval from the Medical Monitor

13. Prior (< 2 years prior to screening) or planned (during the study period) bariatric surgery (e.g., gastroplasty, Roux-en-Y gastric bypass) or reversal or removal of intragastric balloon. Surgery failure < 2 years prior to screening is also exclusionary

14. Other causes of liver disease based on medical history and/or centralized review of liver histology and/or central laboratory results, including but not limited to: alcoholic liver disease, autoimmune disorders (e.g., primary biliary cholangitis [PBC], primary sclerosing cholangitis [PSC], autoimmune hepatitis), drug-induced hepatotoxicity, Wilson disease, clinically significant iron overload, or alpha-1-antitryspin deficiency

15. History of liver transplantation

16. Current or prior history of HCC

17. Current diagnosis of Cushing's syndrome

18. History of significant alcohol consumption for a period of more than 3 consecutive months within 1 year prior to screening

    Note: Significant alcohol consumption is defined as an average exceeding 1 ethanol containing drink/day in female subjects and 2 ethanol containing drinks/day in male subjects

19. Human immunodeficiency virus (HIV) infection

20. Uncontrolled cardiac arrhythmia, or confirmed QT interval corrected using Fridericia's formula (QTcF) > 450 msec for males and > 470 msec for females at screening. Subjects with elevated QTcF (> 450 msec for males and > 470 msec for females) and a cardiac pacemaker may be allowed to participate with prior written approval from the Medical Monitor

    Note: ECG for eligibility assessment may be repeated one time at the Investigator's discretion

21. Myocardial infarction, unstable angina, percutaneous coronary intervention, coronary artery bypass graft, or stroke within 90 days prior to screening

22. Life expectancy less than 2 years

23. Use of any prohibited medication(s) as outlined in Table 5

24. Positive urine drug screen for amphetamines, cocaine, or opiates (e.g., heroin, morphine) at screening. Subjects with a positive urine drug screen due to prescription medication (e.g., opiates, methylphenidate) are eligible if the prescription and diagnosis are reviewed and approved by the Investigator. Subjects on stable methadone or buprenorphine maintenance treatment for at least 180 days prior to screening may be included in the study

25. Unable to safely undergo a liver biopsy

26. Presence of any laboratory abnormality or significant systemic or major illnesses (other than liver disease) that, in the opinion of the Investigator, compromises the subject's ability to safely participate in and complete the study including, but not limited to:

    a. Pulmonary disease, heart failure, renal failure, organ transplantation, serious psychiatric disease, malignancy, history of substance abuse and/or a psychiatric condition requiring hospitalization and/or emergency room visit within 180 days prior to screening

27. Unavailable for follow-up assessment or concern for subject's compliance with the protocol procedures

28. Known hypersensitivity to the study drug, the metabolites, or formulation excipients

4.3.2. Cohort D

Subjects in **Cohort D** who meet *any* of the following exclusion criteria are not to be enrolled in this study:

1. Weight loss > 5% in the 90 days prior to screening until randomization

2. Presence of cirrhosis on liver biopsy (fibrosis stage 4)

3. Type 1 diabetes

4. Unstable T2D defined as:

    a. Insulin dose adjustment > 35% within 30 days prior to screening through randomization

    b. Any history of diabetic ketoacidosis, hyperglycemic hyperosmolar state, and/or acutely decompensated blood glucose control (e.g., thirst, polyuria, weight loss) within 90 days prior to screening

5. Hypoglycemia unawareness, hospitalization due to hypoglycemia, or history of severe hypoglycemia (hypoglycemia requiring outside assistance to regain normal neurologic status) within 90 days prior to screening

6. Subjects with osteoporosis, defined as a T-score of ≤ −2.5 at the femoral neck, total hip, or lumbar spine based on a centrally read DXA scan performed during screening

   Note: A historical DXA scan performed within 90 days prior to screening may be accepted as the screening DXA scan. The historical scan must have been performed on a scanner previously qualified by the central imaging vendor

7. Poorly controlled hypertension (systolic blood pressure > 160 mm Hg, or diastolic blood pressure > 100 mm Hg) at screening

   Note: Vital signs for eligibility assessment may be repeated one time at the Investigator's discretion

8. Current or prior history of decompensated liver disease, including ascites, HE, or variceal bleeding

9. History of pancreatitis

   Note: Subjects with a history of gallstone pancreatitis and subsequent cholecystectomy may be allowed to participate with prior written approval from the Medical Monitor

10. Chronic HBV infection (HBsAg positive) or acute hepatitis A infection (hepatitis A IgM antibody positive). For subjects with positive HBcAb, HBV DNA by quantitative PCR will be required

11. Chronic HCV infection (HCV Ab and HCV RNA positive). Subjects cured of HCV infection < 2 years prior to the Screening visit (based on date of RNA PCR negative confirmation following conclusion of treatment) are not eligible. Asymptomatic subjects with spontaneous resolution of HCV infection (i.e., without RNA PCR negative confirmation following conclusion of treatment) may be allowed to participate with prior written approval from the Medical Monitor

12. Prior (< 2 years prior to screening) or planned (during the study period) bariatric surgery (e.g., gastroplasty, Roux-en-Y gastric bypass) or reversal or removal of intragastric balloon. Surgery failure < 2 years prior to screening is also exclusionary

13. Other causes of liver disease based on medical history and/or centralized review of liver histology and/or central laboratory results, including but not limited to: alcoholic liver disease, autoimmune disorders (e.g., PBC, PSC, autoimmune hepatitis), drug induced hepatotoxicity, Wilson disease, clinically significant iron overload, or alpha-1-antitryspin deficiency

14. History of liver transplantation

15. Current or prior history of HCC

16. History of significant alcohol consumption for a period of more than 3 consecutive months within 1 year prior to screening

    Note: Significant alcohol consumption is defined as an average exceeding 1 ethanol containing drink/day in female subjects and 2 ethanol containing drinks/day in male subjects

17. HIV infection

18. Uncontrolled cardiac arrhythmia, or confirmed QT interval corrected using Fridericia's formula (QTcF) > 450 msec for males and > 470 msec for females at screening. Subjects with elevated QTcF (> 450 msec for males and > 470 msec for females) and a cardiac pacemaker may be allowed to participate with prior written approval from the Medical Monitor

    Note: ECG for eligibility assessment may be repeated one time at the Investigator's discretion

19. Myocardial infarction, unstable angina, percutaneous coronary intervention, coronary artery bypass graft, or stroke within 90 days prior to screening

20. Life expectancy less than 2 years

21. Use of any prohibited medication(s) as outlined in Table 5

22. Positive urine drug screen for amphetamines, cocaine, or opiates (e.g., heroin, morphine) at screening. Subjects with a positive urine drug screen due to prescription medication (e.g., opiates, methylphenidate) are eligible if the prescription and diagnosis are reviewed and approved by the Investigator. Subjects on stable methadone or buprenorphine maintenance treatment for at least 180 days prior to screening may be included in the study

23. Unable to safely undergo a liver biopsy

24. Any contraindication to magnetic resonance imaging (MRI) including, but not limited to: unmanageable claustrophobia, certain metal implants, and inability to fit in MRI machine

25. Presence of any laboratory abnormality or significant systemic or major illnesses (other than liver disease) that, in the opinion of the Investigator, compromises the subject's ability to safely participate in and complete the study including, but not limited to:

    a. Pulmonary disease, heart failure, renal failure, retinopathy, organ transplantation, serious psychiatric disease, malignancy, history of substance abuse and/or a psychiatric condition requiring hospitalization and/or emergency room visit within 180 days prior to screening

26. Unavailable for follow-up assessment or concern for subject's compliance with the protocol procedures

27. Known hypersensitivity to the study drug, the metabolites, or formulation excipients

5. STUDY DRUG(S)

5.1. Randomization, Blinding and Treatment Codes

An Interactive Response Technology (IRT) system will be used for centralized randomization and treatment assignment. Investigative site personnel will obtain the treatment assignment from the IRT system. Subjects and all personnel directly involved in the conduct of the study will be blinded to treatment assignments. Study drugs will be dispensed in a blinded fashion to the subjects.

5.1.1. Procedures for Breaking Treatment Codes

In the event of a medical emergency where breaking the blind is required to provide medical care to the subject, the Investigator may obtain treatment assignment directly from the IRT system for that subject. When possible, the Sponsor recommends, but does not require, that the Investigator contact the Medical Monitor before breaking the blind. Treatment assignment should remain blinded unless that knowledge is necessary to determine subject emergency medical care. The rationale for unblinding must be clearly explained in source documentation and on the electronic case report form (eCRF), along with the date on which the treatment assignment was obtained. The Investigator is requested to contact the Medical Monitor promptly in case of any treatment unblinding, either intentional or accidental.

Blinding of study treatment is critical to the integrity of this clinical trial and, therefore, if a subject's treatment assignment is disclosed to the Investigator, regardless of reason, the subject will have to discontinue study treatment. All subjects will be followed until study completion (i.e., completion of the 30-day Follow-up visit) unless consent to do so is specifically withdrawn by the subject.

The Clinical Research Organization's (CRO's) pharmacovigilance and/or designee may independently unblind cases for expedited reporting of suspected unexpected serious adverse reactions (SUSARs).

5.2. Efruxifermin and Placebo

5.2.1. Formulation, Packaging, and Labeling

Efruxifermin is provided in ISO 2R sterile vials filled with a 1.2 mL (1.0 mL nominal) volume at strengths of 28 or 50 mg/mL. Efruxifermin will be presented as a sterile, colorless to slightly yellow, and preservative-free frozen liquid.

Placebo will be presented in an identical container and fill volume, and packaged the same as EFX.

Refer to the Pharmacy Manual for further instructions on study drug administration.

Study drugs to be distributed to centers in the US and other participating countries shall be labeled to meet applicable requirements of the US Food and Drug Administration (FDA), European Union (EU) Guideline to Good Manufacturing Practice – Annex 13 (Investigational Medicinal Products), and/or other local regulations.

5.2.2. Storage and Handling

Efruxifermin and placebo vials will arrive frozen in individual cartons and must remain in their original packaging and placed immediately in a –20 °C or colder freezer until planned use. The EFX and placebo vials should be stored protected from light in a secure non-frost—free freezer prior to thawing and use. The set point for the freezer should be –20 °C or colder. The set point is a single temperature and should remain constant. Do not refreeze EFX/placebo after thawing.

All vials of study drug must be stored in a securely locked area, accessible only to authorized site staff.

Consideration should be given to handling, preparation, and disposal through measures that minimize drug contact with the body. Appropriate precautions should be followed to avoid exposure when handling.

The site must maintain records of study drug quantities and storage conditions during the study, along with the initials of the person recording the information, including:

- The date and quantity of study drug received
- The date, subject number, and the study drug kit number dispensed
- The date, quantity of used and unused study drug returned
- Storage temperatures

The CRO must be notified if any study drug undergoes a temperature excursion or if the study drug is damaged. Impacted study drug supply should be quarantined and should not be utilized until and unless the Sponsor has advised that it is acceptable to do so.

5.2.3. Dosage and Administration

Refer to the Pharmacy Manual for detailed information regarding the preparation and administration of EFX and placebo.

The study drug will be administered as a weekly 1.0 mL SC injection into the abdomen at the clinic by a qualified staff member or at the subject's home utilizing in-home nursing (**main study** only). The first dose of study drug will be followed by a 15-minute observation period. Subjects should be instructed to watch for symptoms of severe allergic reaction for the 24-48 hours following the initial injection of study drug. The vial ID, date, abdominal quadrant, and time of each injection will be recorded in the eCRF. If injection site reactions are observed, mitigation procedures should be put in place as per local standard of care. Examples of

mitigations include icing the injection site, rotating the quadrant of the abdomen in which the injection is administered, or providing an antihistamine prior to the injection.

Cohort D subjects should continue GLP-1 receptor agonist dosing consistent with their current regimen.

If subjects in the **main study** are unable to attend study visits and/or study centers are unable to accommodate on-site visits due to a pandemic, natural disaster, or other major disruption, procedures will be put in place to allow for utilization of in-home nursing for study drug administration, as necessary.

5.3. Prior and Concomitant Medications

All concomitant medications taken within 30 days prior to screening will be recorded in the eCRFs.

At each study visit, the site will capture all medications taken by the subject since the last visit or during the visit (as applicable). Concomitant medications include prescription medications, nonprescription medications, procedure-related medications, naturopathic therapies, dietary supplements, vitamins, and minerals.

If an increase in the dose of a conditionally allowed therapy (e.g., antidiabetic medication), or addition of a new conditionally allowed therapy is clinically indicated, the Medical Monitor should be contacted prior to implementing any changes. In case a new conditionally allowed therapy needs to be administered during the study, the risk/benefit to the subject should be carefully assessed and consideration given to the timing of any necessary introduction of new medications.

Examples of representative medications that are prohibited or which should be used with caution are listed below in Table 5 and Table 6 for the **main study** and Table 5 and Table 7 for **Cohort D.**

Table 5. Prohibited Concomitant Medications

Drug Class	Agents Disallowed	Guidance
Select chronic immunosuppressants	Chronic systemic[a] corticosteroids	Short courses of systemic corticosteroids (< 14 days) may be allowed if, in the Investigator's opinion, they are not associated with liver steatosis nor clinically relevant
Prescription drugs associated with liver steatosis	Methotrexate, amiodarone, high-dose estrogen, tamoxifen, anabolic steroids, valproic acid	Prohibited from within 365 days prior to screening until the end of the study. Physiological hormone replacement therapy may be allowed prior to screening if, in the Investigator's opinion, they are not associated with liver steatosis or clinically relevant

Drug Class	Agents Disallowed	Guidance
Investigational agents	Active investigational drugs. Prior participation in an experimental vaccine trial (e.g., COVID-19 or other) without sufficient washout or administration of a vaccine under emergency use authorization may be acceptable with prior written approval from the Medical Monitor	30 days prior to Day 1 (or at least 5 half-lives; whichever is longer) through the end of the study

a Intra-articular, topical, nasal, epidural, ophthalmic, or inhaled routes are allowed. Chronic systemic use of corticosteroids equivalent to prednisone > 10 mg/day for ≥ 14 days is not allowed.

Table 6. Main Study Conditionally Allowed Medications

Drug Class	Conditionally Allowed Medications	Guidance
Antioxidants	Vitamin E[a]	Doses ≥ 800 IU/day must be stable for at least 90 days prior to the collection date of the liver biopsy specimen used to assess subject eligibility through randomization. The dose of this medication should remain stable through the end of treatment
Anti-Hyperglycemic Therapy[b]	Thiazolidinediones (including, but not limited to, pioglitazone, rosiglitazone, and lobeglitazone), GLP-1R agonists, or SGLT2 inhibitors[a]	Must be on a stable dose for at least 90 days prior to the collection date of the liver biopsy specimen used to assess subject eligibility through randomization. The dose of this medication should remain stable through the end of treatment
	Insulin	Must be on a stable dose(s) for at least 90 days prior to the collection date of the liver biopsy specimen used to assess subject eligibility through randomization. The dose of this medication should remain stable through the end of treatment, however, EFX is expected to improve insulin sensitivity so dose adjustments may be necessary; subjects on insulin with variable blood glucose levels should be reminded of the symptoms of hypo- and hyper-glycemia and how to correct them
	Metformin	Dose adjustments (but not initiation or discontinuation) of metformin are allowed in the 90 days prior to the collection date of the liver biopsy specimen used to assess subject eligibility. The dose of this medication should remain stable post-biopsy until the end of treatment
	Sulfonylureas or other antidiabetic medication(s)	Must be on a stable dose(s) for at least 90 days prior to the collection date of the liver biopsy specimen used to assess subject eligibility through randomization. The dose of this medication should remain stable through the end of treatment, however, EFX is expected to improve insulin sensitivity so dose adjustments may be necessary; subjects on sulfonylureas or other antidiabetic

Drug Class	Conditionally Allowed Medications	Guidance
		medications with variable blood glucose levels should be reminded of the symptoms of hypo- and hyper-glycemia and how to correct them
	Other weight loss, or lipid-modifying medication(s)	Must be on a stable dose(s) for at least 90 days prior to the collection date of the liver biopsy specimen used to assess subject eligibility through randomization. The dose of this medication should remain stable through the end of treatment

EFX = Efruxifermin; GLP-1R = glucagon-like peptide-1 receptor; SGLT2 = sodium glucose co-transporter 2

a A stable dose is defined as no changes in prescribed dose, initiation of new medications, or discontinuation of existing medications
b Subjects on antidiabetic medications with Type 2 Diabetes will be instructed to monitor their blood glucose at home from Days 2 through 4 after each injection of study drug

Table 7. Cohort D Conditionally Allowed Medications

Drug Class	Conditionally Allowed Medications	Guidance
Antioxidants	Vitamin E[a]	Doses ≥ 400 IU/day must be stable for at least 90 days prior to randomization. The dose of this medication should remain stable through the end of treatment
Anti-Hyperglycemic Therapy[b]	GLP-1R agonists[a]	Must be on a stable dose for at least 90 days prior to screening. The dose of this medication must remain stable through the end of treatment
	Thiazolidinediones (including, but not limited to, pioglitazone, rosiglitazone, and lobeglitazone) and SGLT2 inhibitors[a]	Must be on a stable dose for at least 90 days prior to randomization. The dose of this medication should remain stable through the end of treatment
	Insulin[a]	Must be on a stable dose(s) for at least 90 days prior to randomization. The dose of this medication should remain stable through the end of treatment, however, EFX is expected to improve insulin sensitivity so dose adjustments may be necessary; subjects on insulin with variable blood glucose levels should be reminded of the symptoms of hypo- and hyper-glycemia and how to correct them
	Metformin[a]	Must be on a stable dose(s) for at least 30 days prior to randomization. The dose of this medication should remain stable until the end of treatment
	Sulfonylureas or other antidiabetic medication(s)[a]	Must be on a stable dose(s) for at least 90 days prior to randomization. The dose of this medication should remain stable through the end of treatment, however, EFX is expected to improve insulin sensitivity so dose adjustments may be necessary; subjects on sulfonylureas or other antidiabetic medications with variable blood glucose levels should be reminded of the symptoms of hypo- and hyper-glycemia and how to correct them
	Other weight loss, or lipid-modifying medication(s)[a]	Must be on a stable dose(s) for at least 90 days prior to randomization. The dose

Drug Class	Conditionally Allowed Medications	Guidance
		of this medication should remain stable through the end of treatment

EFX = Efruxifermin; GLP-1R = glucagon-like peptide-1 receptor; SGLT2 = sodium glucose co-transporter 2

a A stable dose is defined as no changes in prescribed dose, initiation of new medications, or discontinuation of existing medications
b Subjects will be instructed to monitor their blood glucose at home from Days 2 through 4 after each injection of study drug

5.4. Study Drug Accountability

The Sponsor or designee will supply the study drugs (EFX and placebo). The site will maintain the following records: temperature excursions, receipt of shipments, dispensation to subjects, and return (and if applicable, destruction) of partially used, or unused study drug.

5.4.1. Study Drug Return or Disposal

At the start of the study, the study monitor will evaluate the study center's study drug disposal procedures and provide appropriate instruction for return or destruction of unused study drug supplies. If the site has an appropriate Standard Operating Procedure (SOP) for drug destruction as determined by the Sponsor, the site may destroy used and unused study drug supplies performed in accordance with the site's (hospital/pharmacy) SOP. If the site does not have acceptable procedures in place for drug destruction, arrangements will be made between the site and the Sponsor (or the Sponsor's representative) for return of unused study drug supplies. A copy of the site's SOP will be obtained for central files. Where possible, study drug will be destroyed at the site. Upon study completion, a copy of the Investigational Drug Accountability records must be filed at the site. Another copy will be returned to the Sponsor. If study drug is destroyed on site, the Investigator must maintain accurate records for all study drug destroyed. Records must show the identification and quantity of each unit destroyed, the method of destruction, and person who disposed of the study drug. All study drug records must be maintained at the site and copies must be submitted to the Sponsor both during and at the conclusion of the study.

6. STUDY PROCEDURES

The study procedures to be conducted during screening and at selected time points are presented in the Schedule of Assessments (Appendix A and Appendix B for the **main study** and Appendix C for **Cohort D**) and described in the text below. Additional procedures deemed necessary as part of standard of care may be performed at the discretion of the Investigator. All missed visits and any procedures performed that are not protocol-specified activities must be documented in the subject's medical record and the appropriate eCRF.

6.1. Study Procedures in Case of Crisis Situations

Additional precautions may be implemented in case of a crisis situation (e.g., COVID-19 pandemic, political strife, natural disaster) to protect subject safety, particularly in the event that a subject is unable to physically attend an onsite visit and/or receive study drug. Investigational study sites must comply with local public health rules and temporary measures may be implemented immediately to comply with local authority's requirements/restrictions. The Regulatory Agency/Institutional Review Board (IRB)/Independent Ethics Committee (IEC) will be notified in parallel of these measures, as appropriate. These additional precautions remain in effect only until the local government, health authorities, and/or other regulatory bodies lift the ban on any lockdown/isolation requirements/guidelines.

Based on assessment of risk, and to ensure subject safety as well as minimize risks to trial integrity, the Sponsor has determined that the following optional off-site study procedures may be performed in case a study participant cannot attend an on-site visit during a crisis situation (e.g., COVID-19 pandemic, political strife, natural disaster):

- Safety assessment via phone or video call, including the subject or proxy answering questions about how the subject is feeling
- Subject's travel to a local lab (if possible) for collection of blood and urine samples and other assessments, with results sent to the Investigator
- Delivery of the study treatment to subject
- Visit to subject's home (e.g., mobile health nursing)

In the event of a crisis situation, these options apply to all on-site study visits except for the screening visit and baseline/randomization visit, which must be performed on-site.

These solutions may be applied depending on the Investigator's judgment of each case and the subject's agreement. The alternative solutions can be implemented in response to a crisis prior to the notification or submission to and approval from regulatory agencies and ethics committees.

Before implementing any of these options for a subject, the site will contact the subject to check whether he/she agrees with the off-site procedures and this will be documented in the subject's source.

The subject will be invited to attend an on-site visit to complete the study procedures as per protocol, as soon as the situation allows.

6.2. Screening and Informed Consent

The Sponsor requires a copy of the study site's written IRB/IEC approval of the protocol, informed consent form (ICF), and all other subject information and/or recruitment material(s), if applicable, before any subject undergoes study-specific activities or procedures. Each subject (or legally acceptable representative) must sign and date the ICF before participating in study-specific activities.

After the ICF is signed, the subject may begin the screening period. Subjects will be screened up to 8 weeks prior to randomization to determine eligibility for participation in the study. The screening period may be extended by 2 weeks under special circumstances, with prior written approval from the Medical Monitor. To minimize risk and overall subject burden, it is recommended that invasive procedures, such as collection of the screening liver biopsy specimen, are performed after confirming that all other study entry criteria have been met. Subjects who screen fail for the **main study** due to fibrosis stage may apply any assessments from the original screening visit that are still within window for potential enrollment into **Cohort D.** Other tests/procedures that do not need to be repeated are listed below.

If there is reasonable belief that an exclusionary result was obtained in error or is transient, a single repeat may be permitted with prior written approval from the Medical Monitor; with the exception of vital signs and ECG, which may be repeated one time at the Investigator's discretion. In addition, subjects who are ineligible (i.e., screen failures) may be rescreened once with prior written approval from the Medical Monitor.

Subjects who are permitted to rescreen must repeat the informed consent process and sign a new ICF. A new screening number will be assigned. The following tests/procedures will not need to be repeated (unless required per Investigator judgment) if available within the appropriate timeframe:

- FibroScan®, if results of a prior FibroScan® examination performed within 90 days prior to the re-screening visit are available
- Liver biopsy,
  - **Main Study**:
    - If the liver biopsy specimen submitted for central reader evaluation during the initial screening visit was collected within 365 days prior to the re-screening date, with results available
    - If a historical liver biopsy specimen collected within 365 days prior to the re-screening date with prior confirmation of fibrosis stage 4 can be submitted successfully for central reader evaluation

— **Cohort D**:

- If a historical liver biopsy specimen collected within 365 days prior to the re-screening date with confirmation of fibrosis stages 1, 2, or 3 from a pathologist is available

- DXA scan, if a previous scan performed within 90 days prior to the re-screening visit is available. The historical scan must have been performed on a scanner previously qualified by the central imaging vendor. For the **main study**, the scanner used for the historical scan must be available for use at post-baseline visits

- ELF score, if results of a prior ELF test performed within 90 days prior to the re-screening visit are available

- For subjects participating in the optional MRE substudy: MRE assessment, if results of a prior MRE assessment performed within 90 days prior to the re-screening visit are available.

After completing the screening period, the subject will be evaluated by the Investigator to confirm eligibility. A subject is considered eligible for randomization when the Investigator determines that all of the eligibility criteria are met. The Investigator will document the decision and the assessment date in the subject's medical record. Screen failure subjects will be entered into the eCRF. Investigators will maintain a screening log of all potential subjects that includes limited information about each subject, including dates and outcome of the screening process (e.g., randomized into study, reason for ineligibility, or withdrawal of consent).

6.3. Demographics and Medical History

Demographic data including sex, age, race, and ethnicity will be collected, as permitted by local law.

The Investigator or designee will collect medical and surgical history that started prior to the time of consent, including information on the subject's concurrent medical conditions. All findings will be recorded on the medical history eCRF.

6.4. Physical Examination

The Investigator or designee will conduct a complete physical examination, or a symptom-driven exam, as outlined in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C). Clinically relevant physical exam findings prior to the first dose of study drug will be recorded on the medical history eCRF page (unless related to study procedure) and clinically significant findings after the first dose of study drug will be recorded as AEs.

At a minimum, the complete physical examination should include assessments of the head and neck, skin, nervous system, lungs, cardiovascular system, abdomen, thyroid, lymph nodes, and extremities.

A symptom-driven physical examination will include assessment of any new subject complaints or changes from baseline.

6.5. Vital Signs, Height, and Weight

6.5.1. Vital Signs

Vital sign measurements will be performed as outlined in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C) and will include systolic blood pressure, diastolic blood pressure, heart rate, and respiration rate.

Temperature will be collected at the Screening and Day 1 visits only. The method for obtaining temperature should be kept consistent across subject visits.

Triplicate vital sign measurements will be performed as outlined in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C) and will include systolic blood pressure, diastolic blood pressure, and heart rate. Triplicate vital signs will be performed in the same arm with ≥ 2 minutes of rest between measurements.

The subject must be in seated or in a semi-recumbent position and in a rested and calm state for ≥ 5 minutes before vital signs are collected. The position selected for a subject should be the same throughout the study and documented on the vital signs eCRF.

6.5.2. Height

Height will be measured without shoes at screening.

6.5.3. Weight

Weight will be measured as outlined in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C) and should be obtained by the same study personnel as much as possible across visits for each subject to minimize variability in the measurements.

Weight should be obtained using a calibrated scale. The subject should be weighed in consistent clothing, as much as possible. On in-clinic dosing days, weight will be collected pre-dose.

6.6. Hip and Waist Circumference – Cohort D Only

Hip and waist circumference will be collected as outlined in the Schedule of Assessments (Appendix C) and should be obtained by the same study personnel as much as possible across visits for each subject to minimize variability in measurements.

Waist circumference should be measured by placing the tape measure around the waist, just above the top of the hip bone. The tape measure should be snug against the skin, but not tight. The tape measure should be parallel to the floor, and not slanted.

Hip circumference should be measured by placing the tape measure around the widest part of the buttocks. The tape measure should be parallel to the floor, and not slanted.

6.7. 12-Lead Electrocardiogram

Electrocardiograms (ECGs) should be obtained after the subject has been resting in a seated, semi-recumbent, or supine position for ≥ 10 minutes and will include QRS, QT, QT interval corrected for heart rate (QTcF), RR, and PR intervals. Each 12-lead ECG should be performed prior to blood draws, dosing (if applicable), or other invasive procedures.

For subjects with a cardiac pacemaker, a QT interval correction formula appropriate for the particular pacing (e.g., Bogossian formula) may be applied per local standard of care. However, elevated QTcF at screening requires Medical Monitor review and approval prior to subject enrollment.

The Investigator or qualified designee will review, sign, and date all ECGs and the results will be entered into the ECG eCRF. The original ECGs will be retained with the subject's source documents. At the request of the Sponsor, a copy of the original ECG will be made available to the Sponsor.

6.8. Model for End-Stage Liver Disease (MELD) Score

Model for End-Stage Liver Disease (MELD) score will be calculated by the central laboratory at the time points specified in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C).

6.9. Child-Pugh Score

Child-Pugh score will be calculated at the time points specified in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C) using the formula in Appendix F.

Physical assessments required to calculate Child-Pugh score are to be completed as part of the complete and symptom-driven physical examinations outlined in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C).

6.10. Abdominal Ultrasound – Main Study Only

Abdominal ultrasounds will be performed and read locally at the time points specified in the Schedule of Assessments (Appendix A and Appendix B) to monitor liver and gallbladder health. Any abnormal findings at baseline in the liver (e.g., hepatic lesion) or gallbladder (e.g., gallstones) should be documented and any new findings during the study should be recorded in the subject's source and the applicable eCRFs.

6.11. FibroScan®

FibroScan® examinations will be performed at the time points specified in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C). Median liver stiffness in kilopascals

(kPa), interquartile range/median value (IQR/M), and success rate (number of valid shots/total number of shots) will be recorded. The median controlled attenuation parameter (CAP) and the interquartile range of CAP values will be recorded from the FibroScan® examinations.

Subjects must fast ≥ 2 hours prior to each FibroScan® assessment. The equipment for performing FibroScan® examinations should be kept consistent across subject visits.

- **Screening, Main Study:** All subjects must complete a FibroScan® examination during the screening period. The FibroScan® inclusion criterion only applies to subjects without a historical liver biopsy, which is defined as a liver biopsy specimen collected ≤ 365 days prior to screening with prior confirmation of fibrosis stage 4 that is available for submission to a central reader for eligibility assessment.

- **Screening, Cohort D:** All subjects must complete a FibroScan® examination during the screening period. The FibroScan® inclusion criterion only applies to subjects without a historical liver biopsy, which is defined as a liver biopsy specimen collected ≤ 365 days prior to screening with confirmation of fibrosis stage 1, 2, or 3 from a pathologist.

If historical values for FibroScan® performed ≤ 90 days prior to the Screening visit are available, then the screening FibroScan® examination does not need to be repeated. The results of the previous scan are to be recorded in the eCRF.

6.12. Liver Biopsy

All reasonable attempts should be made to acquire a needle core liver biopsy specimen of at least 2.0 cm (≥ 10 portal tracts) in length. It is recommended that a 16-gauge or lower needle is used to collect the tissue sample. The subject should remain under observation consistent with the standard of care of that institution after the biopsy procedure.

Main Study

A liver biopsy must be obtained at screening to provide the liver tissue for central reading. A historical biopsy collected within 365 days prior to screening with prior confirmation of fibrosis stage 4 may be accepted as the screening biopsy if the sample is deemed acceptable for interpretation by the central readers. The sample submitted for central reader evaluation must be in line with prior and concomitant medication guidance (refer to Section 5.3).

If the liver biopsy fragment is too small or of bad quality and precludes an adequate read from the central reader, the site may be requested to provide other available slides or prepare new slides from an available block of tissue.

Liver biopsies will be sent to a central laboratory and scanned on a validated imaging scanner and then read by 2 independent, trained pathologists. Both pathologists will read the same screening biopsy slides for eligibility. On-treatment biopsies will be read with screening biopsies in a random, blinded fashion (i.e., not paired) by both pathologists. If significant scoring differences exist between the 2 pathologists for the efficacy analyses, an adjudication meeting

will occur to obtain consensus. The process for scoring of biopsies and minimization of inter- and intra-observer discordance will be further outlined in the Biopsy Management Plan.

If a liver biopsy is performed per standard of care outside of protocol mandated assessments, all possible attempts should be made to submit the biopsy specimen to the central reader for evaluation.

If liver biopsy results are deemed unevaluable by the central reader, additional slides may be provided, or a repeat biopsy may be performed at the discretion of the Investigator.

If a subject refuses to complete the Week 36 liver biopsy, the subject will be discontinued from the study at Week 36. Only a 30-day Follow-up visit is required following discontinuation of study drug.

Cohort D

A historical biopsy collected within 365 days prior to screening with confirmation of liver fibrosis stage 1, 2, or 3 from a pathologist may be accepted as the screening biopsy. If a historical biopsy is not available, a liver biopsy will be performed during the screening period. A pathology report documenting the fibrosis stage must be available to support subject eligibility.

If a liver biopsy is performed per standard of care outside of protocol mandated assessments, all possible attempts should be made to provide the report to the Sponsor.

6.13. Optional Magnetic Resonance Elastography (MRE) Substudy – Main Study Only

The extent of liver stiffness will also be measured by MRE. The optional MRE substudy will be offered at a subset of sites and approximately 30 to 50 subjects who provide their additional specific consent will be enrolled. Subjects who have contraindications to magnetic resonance imaging (including, but not limited to: unmanageable claustrophobia, certain metal implants, and inability to fit in an MRI machine) should be excluded from the substudy. Subjects must fast ≥ 4 hours prior to each MRE assessment.

All subjects participating in the optional MRE substudy will undergo imaging at the timepoints specified in the Schedule of Assessments (Appendix A and Appendix B).

The MRE assessment at the Screening visit should be performed 1-14 days prior to the Day 1 visit and after confirming that eligibility criteria have been met. A historical MRE assessment may be accepted for re-screens (refer to Section 6.2 for details).

The MRE images will be analyzed at a central imaging facility. Refer to the MRE Imaging Manual for image acquisition guidelines.

6.14. Magnetic Resonance Imaging - Proton Density Fat Fraction (MRI-PDFF) – Cohort D Only

The degree of liver steatosis will be measured by MRI-PDFF. Subjects who have contraindications to magnetic resonance imaging (including, but not limited to: unmanageable claustrophobia, certain metal implants, and inability to fit in an MRI machine) as outlined in the exclusion criteria should be excluded from **Cohort D**. Subjects must fast ≥ 4 hours prior to each MRI-PDFF assessment.

All subjects participating in **Cohort D** will undergo imaging at the timepoints specified in the Schedule of Assessments (Appendix C).

The MRI-PDFF assessment at the Screening visit should be performed 1-14 days prior to the Day 1 visit and after confirming that eligibility criteria have been met.

The MRI-PDFF images will be analyzed at a central imaging facility. Refer to the Imaging Manual for MRI-PDFF imaging guidelines.

6.15. Dual-energy X-ray Absorptiometry (DXA) Scan

A DXA scan will be performed to measure bone loss and should include lumbar spine, femoral neck, and total hip with subjects in the standard (supine) position.

At the DXA time points in the **main study**, total body composition may also be collected, where available, to determine body fat distribution.

- **Main Study:** A historical DXA scan performed within 90 days prior to screening may be accepted as the screening DXA scan for the **main study**. The historical scan must have been performed on a scanner previously qualified by the central imaging vendor that is available for use at post-baseline visits.

- **Cohort D:** A historical DXA scan performed within 90 days prior to screening may be accepted as the screening DXA scan for **Cohort D**. The historical scan must have been performed on a scanner previously qualified by the central imaging vendor.

Refer to the Imaging Manual for DXA imaging acquisition guidelines.

6.16. Chronic Liver Disease Questionnaire-Nonalcoholic Steatohepatitis (CLDQ-NASH) – Main Study Only

The CLDQ-NASH asks questions on a 7-point Likert scale with 1 representing "all of the time" and 7 representing "none of the time." Higher scores indicate better health-related quality of life in subjects with chronic liver disease. The questionnaire collects data on 36 items grouped into 6 domains (abdominal symptoms, activity/energy, emotional health, fatigue, systemic symptoms, and worry) with a 2-week recall period.

It is recommended that the questionnaire be completed prior to any study procedures being performed and prior to the subject seeing a health care provider. If the baseline questionnaire is missed at the Day 1 visit, the questionnaire should not be collected at the remaining time points.

6.17. Work Productivity and Activity Impairment for Nonalcoholic Steatohepatitis (WPAI-NASH) – Main Study Only

The WPAI-NASH is a questionnaire developed to measure impairments in work activities in subjects with NASH.

The questionnaire consists of 6 questions (currently employed, work time missed due to NASH, work time missed due to other reasons, hours actually worked, degree NASH affected productivity while working [0—10 numeric rating scale (NRS); with 0 indicating no effect and 10 indicating NASH completely prevented the subject from working], and degree NASH affected productivity in regular unpaid activities [0—10 NRS; with 0 indicating no effect and 10 indicating NASH completely prevented the subject's daily activities]). The recall period for questions 2 through 6 is 7 days.

Four main outcomes (expressed in percentages) can be obtained from the WPAI- NASH: percentage of work time missed due to NASH, percentage of impairment due to NASH, percentage of overall work impairment due to NASH, and percentage of activity impairment due to NASH. Note that for subjects who did not work during the 7 days covered by the WPAI-NASH, the percent overall work impairment will be equal to the percent of work time missed due to NASH.

It is recommended that the questionnaire be completed prior to any study procedures being performed and prior to the subject seeing a health care provider. If the baseline questionnaire is missed at the Day 1 visit, the questionnaire should not be collected at the remaining time points.

6.18. Lifestyle Guidance

All subjects will receive counseling regarding lifestyle including the maintenance of a healthy diet and physical activity at the Screening visit. Counseling should include lifestyle recommendations developed by the American College of Cardiology/American Heart Association (Arnett 2019):

1) All adults should consume a healthy diet that emphasizes the intake of vegetables, fruits, nuts, whole grains, lean vegetable or animal protein, and fish and minimizes the intake of trans fats, red meat and processed red meats, refined carbohydrates, and sweetened beverages. For adults who are overweight and obese, counseling and caloric restriction are recommended for achieving and maintaining weight loss.

2) Adults should engage in at least 150 minutes per week of accumulated moderate-intensity physical activity or 75 minutes per week of vigorous-intensity physical activity.

3) For adults with type 2 diabetes mellitus, lifestyle changes, such as improving dietary habits and achieving exercise recommendations, are crucial.

At each subsequent visit, subjects will be asked if there has been a significant change in diet or physical activity. Changes will be documented in the eCRF.

6.19. Laboratory Assessments

6.19.1. Laboratory Sample Collection, Storage, and Shipping

Blood, saliva, and urine samples will be collected according to the Schedule of Assessments (Appendix A, Appendix B, and Appendix C), Table 8, and as described in the following text. Samples will be sent to the central laboratory for storage, processing, analysis, and/or reporting. Validated assays will be used for analysis, where appropriate.

At visits where indicated in the Schedule of Assessments (Appendix A), subjects in the **main study** will be given take-home supplies to complete the required 24-hour urine collections.

Samples may be analyzed for the tests outlined in this protocol and for any additional tests necessary to ensure subject safety. These may include, but are not limited to, investigation of unexpected results and incurred sample reanalysis.

At visits where indicated, samples should be collected in the morning following an overnight fast (no food or drink, except water) of at least 8 hours.

Subjects in the **main study** who do not have a historical liver biopsy specimen require a screening ELF score. If a historical value for an ELF test performed ≤ 90 days prior to the Screening visit is available, then the screening ELF score does not need to be repeated. The result of the previous test is to be recorded in the eCRF.

The details of sample handling and shipment instructions will be provided by the central laboratory in a separate laboratory manual.

Table 8. Laboratory Assessments

Hematology	Chemistry	Urine	PK and Immunogenicity
Hematocrit Hemoglobin Platelet count Red blood cell count White blood cell count Differentials (absolute and percentage), including: Lymphocytes Monocytes Neutrophils Eosinophils Basophils	Alkaline phosphatase Aspartate aminotransferase Alanine aminotransferase Gamma-glutamyl transferase Total bilirubin Direct bilirubin Total protein Albumin Bicarbonate Blood urea nitrogen Calcium Chloride Serum creatinine Glucose[a] Phosphorus Magnesium Potassium Sodium Amylase Lipase[b] Creatine kinase Hemoglobin A1c 3 Hydroxybutyrate Bile acids Uric acid Lactic acid dehydrogenase High-sensitivity C-reactive protein Estimate glomerular filtration rate	Urine drug screen Amphetamines Cocaine Opiates (i.e., heroin, morphine) Urinalysis 24-hour urine creatinine[c,h] 24-hour urinary free cortisol[c,h]	EFX PK Anti-drug (EFX) antibody Neutralizing anti-drug (EFX) antibody
		Serology	**Biomarkers**
		Hepatitis A immunoglobulin M antibody Hepatitis B surface antigen Hepatitis B core antibody Hepatitis B virus DNA (reflex) Hepatitis C antibody Hepatitis C virus RNA (reflex) HIV	Fibrosis Biomarkers ELF Pro-C3 C3M Bone Biomarkers P1NP CTX-1[a] Bone-specific alkaline phosphatase Vitamin D Parathyroid hormone Lipid Metabolism Biomarkers Apolipoprotein B Apolipoprotein C3 Insulin Sensitivity and Glycemic Control Biomarkers Insulin[a] Adiponectin C-Peptide[a] HOMA-IR[a]
Coagulation	**Lipoproteins**	**Pregnancy[d]**	**Other**
Prothrombin time Activated partial thromboplastin time International normalized ratio Plasminogen activator inhibitor-1 Fibrinogen	Lipoprotein(a) Lipid Panel[a] Total cholesterol High-density lipoprotein cholesterol Low-density lipoprotein cholesterol Very low-density lipoprotein cholesterol (calculated) Non-high—density lipoprotein cholesterol Triglycerides	*In females of childbearing potential:* Serum pregnancy Urine pregnancy	Endogenous FGF21[a] Phosphatidylethanol[h] Optional Genetic Substudy (e.g., PNPLA3) Storage samples[e] Serum storage samples Plasma storage samples Optional PBMC Substudy[h] AAT[h] Ceruloplasmin[f,h] Hormones Salivary cortisol[g] Thyroid-stimulating hormone

			Insulin-like growth factor-1[h] Free T4 Glucagon[i]

AAT = alpha-1-antitrypsin; C3M = type III collagen; CTX-1 = C-telopeptide of type I collagen; EFX = Efruxifermin; ELF = Enhanced Liver Fibrosis panel; FGF21 = Fibroblast growth factor 21; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; P1NP = procollagen type 1 N pro-peptide; PBMC = peripheral blood mononuclear cell; PK = pharmacokinetics; PNPLA3 = patatin-like phospholipase domain-containing protein 3; Pro-C3 = pro-peptide of type 3 procollagen

a Samples to be collected in the morning following an overnight fast of at least 8 hours
b Reflex lipase testing is performed in subjects with total amylase > 1.5 upper-limit of normal
c The 24-hour urine collection for the Screening visit should be initiated 1-7 days prior to the Day 1 visit. The window may be extended with prior written approval from the Sponsor
d For female subjects of childbearing potential only. Serum pregnancy test at screening and urine pregnancy test at all other time points
e Serum and plasma storage samples to be collected and banked for possible additional clinical testing
f Additional testing may be performed at screening to rule out Wilson disease in subjects with low ceruloplasmin values
g Salivary cortisol sample at each protocol specified visit should be time matched (± 3 hours) to the Day 1 visit collection
h Samples to be collected from subjects in the **main study** only
i Samples to be collected from subjects in **Cohort D** only

6.19.2. Pharmacokinetic Assessments

Pharmacokinetic samples will be collected at time points specified in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C).

6.19.2.1. Optional Pharmacokinetic Substudy – Main Study Only

Approximately 18 subjects who provide their additional specific consent will participate in the optional PK substudy. Serial blood samples for PK analysis will be collected relative to the first dose of study drug on Day 1. To allow for subject flexibility, not all blood samples will be mandatory.

Table 9. Optional Pharmacokinetic Substudy – Day 1

Visit	Day 1	Day 2	Day 3	Day 4	Day 5	Day6
Hour[a]		24 hours	48 hours	72 hours	96 hours	120 hours
Serial EFX PK		X	X	X	X	X

EFX = Efruxifermin; PK = pharmacokinetic

a Samples should be collected as close as possible to the nominal time

In addition, sparse PK samples will be collected 48 to 96 hours post-dose following any 2 visits between Week 6 and Week 12, inclusive. Triplicate vital signs will be obtained prior to each sparse PK collection and as described in Section 6.5.1.

Table 10. Optional Pharmacokinetic Substudy – Week 6 to Week 12

Visit	Visit 1 (between Week 6 to Week 12, inclusive)	Visit 2 (between Week 6 to Week 12, inclusive)
Hour	**48 to 96 hours post-dose**	**48 to 96 hours post-dose**
Sparse EFX PK 1	X	
Sparse EFX PK 2		X
Triplicate vital signs[a]	X	X

EFX = Efruxifermin; PK = pharmacokinetic

a Triplicate vital signs will be performed prior to each sparse PK sample collection

6.19.3. Optional Genetic Substudy

For subjects who provide their additional specific consent for the optional genomic substudy, a blood sample will be collected on Day 1.

6.19.4. Optional Peripheral Blood Mononuclear Cell Substudy – Main Study Only

Approximately 75 subjects who provide their additional specific consent will participate in the optional peripheral blood mononuclear cell (PBMC) substudy. Blood samples will be collected on Day 1, Week 4, Week 20, Week 36, Week 52, Week 96, Early Termination, and 30-day Follow-up Visit.

6.19.5. Immunogenicity Assessments

Blood samples will be obtained for detection of ADA against EFX as outlined in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C).

In the event of a positive anti-EFX antibody detection, a neutralizing anti-drug antibody (NAb) assay will be run for the sample time point with the positive result.

Subjects who test positive for binding antibodies to EFX at the final scheduled study visit will be required to return for additional follow-up testing. This testing will occur once the site has been notified of the positive result and approximately every 3 months thereafter until: (1) antibodies are no longer detectable or (2) the subject has been followed for a period of at least 6 months (± 2 weeks) after the last administration of EFX. All follow-up results, both positive and negative, will be communicated to the sites. More frequent testing (e.g., every month) or testing for a longer period of time may be requested in the event of safety-related concerns. Follow-up testing is not required where it is established that the subject did not receive EFX.

6.19.6. Biomarkers

The biological specimens collected in this study will be used to evaluate the association of exploratory systemic biomarkers with study drug response, including efficacy and/or AEs and to increase knowledge and understanding of the biology of how EFX affects subjects with NASH. Biological specimens may also be used to increase the knowledge of diseases such as NAFLD,

liver fibrosis, inflammatory diseases, bone turnover, and/or the validation of a companion diagnostic, if applicable. The specific analyses will include, but will not be limited to, the biomarkers and assays described in Table 8. As biomarker science is a rapidly evolving area of investigation, and AEs in particular are difficult to predict, it is not possible to specify prospectively all tests that will be performed on the specimens provided. The testing outlined is based upon the current state of scientific knowledge and may be modified during or after the end of the study to remove tests no longer indicated and/or to add new tests based upon the newest technologies.

Samples will be destroyed no later than 15 years after the end of the study. The specimen storage period will be in accordance with the IRB/IEC approved ICF and applicable laws (e.g., health authority requirements).

6.19.7. Sample Storage

Biological samples collected during the study will be frozen and stored. The stored samples may be used for future clinical laboratory testing to provide additional clinical data. No human genetic testing will be performed without expressed consent of study subjects. At the conclusion of the study, these samples may be retained in storage by the Sponsor for a period of up to 15 years or per local guidelines.

6.20. Subject Retention

Given the burden of study assessments, subject commitment, and potential commercial availability of treatments for subjects with NASH, subject retention in a long-term, placebo-controlled clinical outcomes study may be challenging. The objective of the subject retention plan is to describe the activities and tactics that may be implemented with the goal of retaining subjects on treatment and in follow-up through the clinical outcomes portion of the study. To achieve this objective, the following types of approaches may be applied:

Site Support and Tactics

The Sponsor and its designee(s) will aim to provide sites with support and tools to encourage behaviors that optimize subject engagement, retention, and compliance:

- Clinical Research Organization staff and Sponsor staff will maintain regular contact (i.e., telephone calls or in-person, as available) with sites to specifically discuss retention and compliance at each site and to exchange and share experiences and best practices
- Site newsletters emphasizing retention tactics and metrics will be employed. Retention Training Webinars will be held to reinforce progress and share ideas across sites, as applicable
- Site support materials will be provided to motivate and assist sites in engaging in subject retention activities

- Clinical Research Organization staff and Sponsor staff will continue to reinforce key behaviors with site staff such as: encouraging relationship-building with subjects, conducting regular check-in phone calls or video-capable telehealth engagements with subjects, office visit reminder phone calls to subjects as well as ensuring that the Principal Investigator (or qualified designee) is present at study visits, talking directly to subjects and re-enforcing the importance of the subject's participation in the study

- Provide support to minimize subjects lost to follow-up through use of a third-party investigative service(s) to search for subject location and contact information, vital status research, and death certificate retrievals

Details of the materials and tools to be utilized are outlined in the Subject Recruitment and Retention Plan.

6.21. Criteria for Discontinuation of Study Drug

Study drug must be discontinued in the following instances:

- Liver-related clinical event that warrants discontinuation as determined by the Hepatic Events Adjudication Committee

- Subject develops an SAE consisting of a serious hypersensitivity reaction to study drug

- Subject experiences diarrhea that meets the criteria in Section 6.23.7 for discontinuation

- Subject experiences signs or symptoms of hyper- or hypo- thyroidism that are accompanied by abnormalities detected in thyroid function tests

- Subject experiences a Grade IV Common Terminology Criteria for Adverse Events (CTCAE) or meets the criteria for drug-induced liver injury (DILI) described in Section 6.23.9

  — The Data Monitoring Committee (DMC) should perform a causality assessment. The study drug may be restarted if the DMC concludes that the AE or laboratory abnormalities were not related to study drug, the AE has resolved, laboratory abnormalities have returned to baseline, and the subject is amenable to close clinical follow-up

  — If, after re-challenge, a subject has a second AE or recurrent elevations (even mild or minimal) of total bilirubin, ALT, or AST as defined in Section 6.23.9, study drug should be discontinued permanently

- Unacceptable toxicity or toxicity that, in the judgment of the Investigator or the Sponsor, compromises the ability to continue study-specific procedures or is considered to not be in the subject's best interest

- Significant subject noncompliance, including refusal to complete the Week 36 liver biopsy
- Significant protocol violation that impacts subject safety
- Pregnancy during the study; refer to Appendix D
- Discontinuation of the study at the request of the Sponsor, a regulatory agency, or an IRB/ IEC

In addition to the above, study drug must be discontinued in the following instance for subjects enrolled in the **main study**:

- If 24-hour urinary free cortisol result is > 300 micrograms per day, interrupt study drug and conduct repeat testing. If the elevation is confirmed upon repeat testing, the subject should be discontinued permanently
  - The Investigator should refer the subject to an endocrinologist for further evaluation, including adrenocorticotropic releasing hormone testing, and for appropriate management of Cushing's syndrome.

6.22. Interruption of Study Drug

If dosing is interrupted (i.e., as a result of an AE or intercurrent illness that would affect assessments of clinical status to a significant degree), every attempt should be made to keep the subject in the study and continue to perform the required study-related procedures. Discussion with the Medical Monitor is recommended. If this is not possible or acceptable to the subject or Investigator, the subject may be withdrawn from the study.

6.23. Adverse Events and Toxicity Management

6.23.1. Adverse Events

An AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study drug, whether or not related to the study drug. Adverse events may also include pre- or post-treatment complications that occur as a result of protocol specified procedures, lack of efficacy, overdose, drug abuse/misuse reports, or occupational exposure. Pre-existing events that increase in severity or change in nature during or as a consequence of participation in the clinical study will also be considered AEs.

Adverse events that occur after the first dose of study drug, during study treatment, and through the safety follow-up period will be documented on the AE eCRF. In addition, after the informed consent, but prior to initiation of study drug, AEs related to protocol-mandated procedures will be recorded on the AE eCRF. All other untoward medical occurrences observed after informed consent and prior to the first dose of study drug, including exacerbation or changes in medical history, will be captured on the medical history eCRF. The Investigator will assess the AE severity and relationship of the AE to study drug and/or study-related procedures. The

Investigator will treat the subject as medically required. Details on recording and reporting AEs are provided in Section 6.23.5.

Laboratory values that are outside the laboratory reference range of normal should be reported as AEs only if considered clinically significant by the Investigator.

Clinical judgment of the Investigator should be used to determine whether a subject is to be withdrawn due to an AE. In the event the subject requests to withdraw from study-related treatment or the study due to an AE, the subject should be followed for the safety follow up period as outlined in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C).

All subjects experiencing AEs, including clinically significant abnormal laboratory values, whether or not associated with use of the study drug, must be monitored until the condition returns to normal, returns to the subject's baseline, the Investigator determines the AE has reached a stable outcome and is no longer clinically significant, or the subject is considered lost to follow up.

6.23.1.1. Severity

All AEs, both serious and non-serious, will be assessed for severity using the CTCAE v5.0. The CTCAE includes unique clinical descriptions of AEs that are categorized by anatomy and/or pathophysiology and is provided in the Site Operations Manual.

The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE (including abnormal laboratory values) based on the guidelines provided within the scale. For any AEs not covered by the CTCAE, the CTCAE definitions for severity will be used (Table 11).

Table 11. Grading of Adverse Event Not Covered by the CTCAE

Grade	Adjective	Description
Grade 1	Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate	Minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL[a]
Grade 3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]
Grade 4	Life-threatening	Life-threatening consequences; urgent intervention indicated
Grade 5	Fatal	Death related to AE

ADL = activities of daily living; AE = adverse event

a Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

b Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_5x7.pdf

6.23.1.2. Relationship

The Investigator or qualified sub-Investigator is responsible for assessing the relationship to study drug using clinical judgment and the following considerations:

- Not related: all AEs should be considered related unless the AE is clearly unrelated to study drug or clearly due to the subject's underlying disease. For SAEs, an alternative causality must be provided (e.g., preexisting condition, underlying disease, intercurrent illness, or concomitant medication)
- Related: There is reasonable possibility that the event may have been caused by the study drug

Ineffective treatment should not be reported as an AE.

The relationship to study-related procedures (e.g., invasive procedures such as venipuncture or biopsy) should be assessed using the following considerations:

- Not related: Evidence exists that the AE has an etiology other than the study procedure
- Related: The AE occurred as a result of study procedures.

6.23.2. Study Stopping Rules

The DMC will be convened based on emerging safety data if any of the following occur:

- One or more subjects develop the same treatment-emergent Grade 5 AE or lab abnormality (confirmed by repeat testing) deemed related to EFX
- Two or more subjects develop the same treatment-emergent Grade 4 or higher AE or lab abnormality (confirmed by repeat testing) deemed related to the same dose level of EFX
- Three or more subjects develop the same treatment-emergent Grade 3 or higher AE or lab abnormality (confirmed by repeat testing) deemed related to the same dose level of EFX

The DMC will assess the causality and clinical relevance of the AEs or lab abnormalities and recommend to either halt study dosing for all subjects, pause randomization, or take no action. If randomization or dosing is paused, it may be resumed (with or without modifications) only after further review of all available safety data and agreement by the appropriate regulatory agencies.

6.23.3. Overdose

An overdose is any dose of study drug given to a subject that exceeds the protocol specified dose by 10% or more. In the event of an overdose-associated AE, appropriate supportive therapy should be initiated according to the subject's signs and symptoms.

Any overdose, with or without associated AEs, will be promptly reported to the Sponsor and recorded as non-compliance on the eCRF. Adverse events associated with overdose should be reported on relevant AE/SAE sections in the eCRF.

6.23.4. Serious Adverse Events

6.23.4.1. Definition

An SAE is defined by federal regulation as any AE occurring at any dose that results in any of the following outcomes: death, life-threatening AE, in-patient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect.

Serious adverse events that occur after the first dose of study drug, during study treatment, and through the safety follow-up period will be documented on the AE eCRF. In addition, after the informed consent, but prior to initiation of study drug, SAEs related to protocol-mandated procedures will be recorded on the AE eCRF. All other untoward serious medical occurrences observed after informed consent and prior to the first dose of study drug, including exacerbation or changes in medical history, will be captured on the medical history eCRF.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject, and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

A life-threatening AE is one that, in the view of the Investigator, places the subject at immediate risk of death from the reaction as it occurred.

An unexpected AE is any event for which the specificity or severity is not consistent with the AE profile in the current EFX IB.

All SAEs, regardless of cause(s) or relationship to study drug, must be reported immediately to the study Sponsor and/or designee.

6.23.5. Reporting Adverse Events

6.23.5.1. Reporting Procedures for Non-Serious Adverse Events

The Investigator is responsible for ensuring that all AEs observed by the Investigator (or designee) or reported by the subject are recorded in the AE eCRF.

The Investigator will assign the following AE attributes:

- AE diagnosis or syndrome(s), if known (if not known, signs or symptoms)

- Dates of onset and resolution (if resolved)
- Severity
- Relatedness to study drug or study-related procedures
- Action taken with study treatment

Follow-up of non-serious AEs will continue until 30 days after the last dose of study drug and/or until a definitive outcome (e.g., resolved, resolved with sequelae, lost to follow-up) is achieved.

When a subject is withdrawn from the study because of a non-serious AE, the Sponsor and/or designee must be notified by e-mail or phone within 48 hours.

6.23.5.2. Reporting Procedures for Serious Adverse Events

The Investigator is responsible for ensuring that all SAEs observed by the Investigator (or designee) or reported by the subject are promptly assessed and reported. The Investigator must assess whether the SAE is related to study drug or any study-related procedure.

The procedures for reporting SAEs are as follows:

Initial Reports

- All SAEs must be reported to Medpace Clinical Safety within 24 hours of the knowledge of the occurrence. After the protocol-specified reporting window, any SAE that the Investigator considers related to study must be reported to Medpace Clinical Safety or the Sponsor/designee
- To report the SAE, complete the SAE form electronically in the electronic data capture (EDC) system for the study, indicating SAE criteria has been met. When the form is completed, Medpace Safety personnel will be notified electronically by the EDC system and will retrieve the form. If the event meets serious criteria and it is not possible to access the EDC system, send an email to Medpace Clinical Safety at medpace-safetynotification@medpace.com or call the Medpace SAE hotline (phone number listed below). Fax/email the completed paper SAE form to Medpace Clinical Safety at medpace-safetynotification@medpace.com, +1-866-336-5320, **or** +1-1-513-570-5196 within 24 hours of awareness

NOTE: When the EDC system becomes available, the SAE information must be entered within 24 hours of the system becoming available.

Follow-up Reports

- The Investigator must continue to follow the subject until the SAE has subsided or until the condition becomes chronic in nature, stabilizes (in the case of persistent impairment), or the subject dies

- Within 24 hours of receipt of follow-up information, the Investigator must update the SAE form electronically in the EDC system for the study, and submit any supporting documentation (e.g., subject discharge summary or autopsy reports) to Medpace Clinical Safety via fax or e-mail. If it is not possible to access the EDC system, refer to the procedures outlined above for initial reporting of SAEs

- The Sponsor or/or designee may request additional information from the Investigator to ensure the timely completion of accurate safety reports

The Investigator must take all therapeutic measures necessary for resolution of the SAE. Any medications or therapies necessary for treatment of the SAE must be recorded in the SAE form and the concomitant medication eCRF.

Follow-up of SAEs will continue through 30 days after the last dose of study drug and/or until a definitive outcome (e.g., resolved, resolved with sequelae, lost to follow-up, fatal) is achieved.

If a subject becomes pregnant during the study or within the period defined in Appendix D, the Investigator is to stop dosing with study drug(s) immediately and the subject should be withdrawn from the study treatment. Early termination procedures and safety follow-up should be implemented at that time. Assessments that are contraindicated during pregnancy should not be performed.

A pregnancy is not considered to be an AE or SAE; however, it must be reported to Medpace Clinical Safety within 24 hours of knowledge of the event. Medpace Clinical Safety will then provide the Investigator/site the Exposure In Utero (EIU) form for completion. The Investigator/site must complete the EIU form and fax/email it back to Medpace Clinical Safety.

If the female partner of a male subject becomes pregnant while the subject is receiving study drug or within the follow up period defined in Appendix D, the Investigator should notify Medpace Clinical Safety as described above.

The pregnancy should be followed until the outcome of the pregnancy, whenever possible. Once the outcome of the pregnancy is known, the follow-up EIU form should be completed and faxed/emailed to Medpace Clinical Safety. If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (i.e., postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly), the Investigator should follow the procedures for reporting an SAE.

Medpace Clinical Safety Contact Information:

E-mail: medpace-safetynotification@medpace.com

Medpace SAE Hotline – US

- Telephone: +1-800-730-5779, option "3" **or** +1-513-579-9911, option "3"

- Facsimile: +1-866-336-5320 **or** +1-1-513-570-5196

Some SAEs will qualify for reporting to the US FDA, as applicable, via the MedWatch/Council for International Organizations of Medical Sciences (CIOMS) reporting system in accordance with FDA and other applicable regulations. The Sponsor, or its designee, will report SAEs and/or SUSARs, as required, to regulatory authorities and Investigators in compliance with all reporting requirements, local regulations, and GCP.

Depending on relevant local legislation or regulations, including the applicable US FDA Code of Federal Regulations (CFR), the EU Clinical Trials Directive (2001/20/EC) and relevant updates, and other country-specific legislation or regulations, the Sponsor may be required to expedite to worldwide regulatory agencies reports of SAEs, serious adverse drug reactions, or SUSARs. In accordance with the EU Clinical Trials Directive (2001/20/EC), the Sponsor or a specified designee will notify worldwide regulatory agencies and the relevant IRB/IEC in concerned Member States of applicable SUSARs as outlined in current regulations.

The Investigator will notify the appropriate IRB/IEC of SAEs occurring at the study site and other AE reports received from the Sponsor, in accordance with local procedures and statutes. The Investigator (or designee) at each study site is responsible for submitting Investigational New Drug (IND) safety reports (initial and follow-up) and other safety information (e.g., revised IB) to the IRB/IEC and for retaining a copy in the study files.

6.23.6. Adverse Events of Special Interest

The Investigator will monitor each subject for clinical and laboratory-evidence for predefined AESIs throughout the subject's participation in this study. The Investigator should treat the AESI according to local standard of care. The following conditions will be monitored as AESIs during this study:

- Injection site reactions
- Diarrhea
- Hypoglycemia
- Neurological and psychiatric events
- Drug-induced liver injury

Drug-induced liver injury will be monitored and managed according to Section 6.23.9.

During the course of the study, additional AESIs may be identified by the Sponsor. The Investigator will assess and record in detail any additional information for AESIs on the AE eCRF form.

Subjects who experience gastrointestinal AEs while on study should be managed according to local standard of care, including concomitant medications to manage symptoms.

6.23.7. Clinical Laboratory Abnormalities and Other Abnormal Assessments as Adverse Events or Serious Events

Laboratory abnormalities are usually not recorded as AEs or SAEs, however, laboratory abnormalities (e.g., clinical chemistry, hematology) independent of the underlying medical condition that require medical or surgical intervention or lead to study drug interruption or discontinuation must be recorded as an AE, as well as an SAE, if applicable. In addition, laboratory or other abnormal assessments (e.g., ECG, X-rays, vital signs) that are associated with signs and/or symptoms must be recorded as an AE or SAE if they meet the definition of an AE (or SAE) as described in Section 6.23.1 and 6.23.4. If the laboratory abnormality is part of a syndrome, record the syndrome or diagnosis (e.g., anemia) not the laboratory result (i.e., decreased hemoglobin).

Severity should be recorded and graded according to the CTCAE v5.0. For AEs associated with laboratory abnormalities, the event should be graded based on the clinical severity in the context of the underlying conditions; this may or may not be in agreement with the grading of the laboratory abnormality.

The Investigator, in consultation with the Medical Monitor, should refer a subject enrolled in the **main study** to an endocrinologist for further evaluation if any of the following laboratory abnormalities are observed and are accompanied by related symptoms or risk factors:

- Abnormal insulin-like growth factor-1 (IGF-1) result, confirmed by repeat testing, where the value has changed significantly from baseline
- 24-hour urinary free cortisol result > ULN, confirmed by repeat testing, where the value has increased significantly from baseline.

Refer to Section 6.21 for guidance on discontinuing study drug due to an adverse event and/or laboratory abnormality.

6.23.7.1. Toxicity Management

Any questions regarding toxicity management should be directed to the Medical Monitor.

Grade 1 and 2 Laboratory Abnormality or Clinical Event

Continue study drug at the discretion of the Investigator.

Grade 3 Laboratory Abnormality or Clinical Event

- For Grade 3 clinically significant laboratory abnormality or clinical event, study drug may be continued if the event is considered to be unrelated to study drug
- For a Grade 3 clinical event, or clinically significant laboratory abnormality confirmed by repeat testing, that is considered to be related to study drug, study drug should be withheld until the toxicity returns to ≤ Grade 2

- If a laboratory abnormality recurs to ≥ Grade 3 following re-challenge with study drug and is considered related to study drug, then study drug should be permanently discontinued, and the subject managed according to local practice. Recurrence of laboratory abnormalities considered unrelated to study drug may not require permanent discontinuation

Grade 4 Laboratory Abnormality or Clinical Event

- For a Grade 4 clinical event or clinically significant Grade 4 laboratory abnormality confirmed by repeat testing, the DMC should perform a causality assessment. The study drug may be restarted if the DMC concludes that the AE or laboratory abnormality were not related to study drug, the AE has resolved and/or the laboratory abnormality has returned to baseline, and the subject is amenable to close clinical follow-up

- If a laboratory abnormality recurs to ≥ Grade 4 following re-challenge with study drug and is considered related to study drug, then study drug should be permanently discontinued, and the subject managed according to local practice. Recurrence of laboratory abnormalities considered unrelated to study drug may not require permanent discontinuation

- If, after re-challenge, a subject has a second serious AE or recurrent elevations (even mild or minimal) of total bilirubin, ALT, or AST, as defined in Section 6.23.9, study drug should be discontinued permanently

6.23.8. Adjudication Committees

External adjudication committees will be established to adjudicate cases that meet protocol-defined criteria for liver-related events, and for cardiovascular-related events for the **main study**. If there are any liver-related or cardiovascular-related events for **Cohort D**, they will also be reviewed by the respective adjudication committees.

6.23.8.1. Hepatic Events Adjudication Committee – Main Study Only

A key objective of this study is to prevent complications of cirrhosis. Clinical events that constitute the clinical outcomes endpoint include:

1) Events of hepatic decompensation including:

   a) Clinically apparent ascites requiring treatment, refractory ascites, or spontaneous bacterial peritonitis

   b) Hepatic encephalopathy of Grade 2 or above (according to the West Haven criteria defined in Appendix E) requiring treatment

   c) Portal hypertension-related upper gastrointestinal bleeding identified by endoscopy and requiring hospitalization, including events of bleeding from esophageal varices, gastric varices, and portal hypertensive gastropathy

d) Child-Pugh score > 6 on 2 consecutive occasions at least 14 days apart, unless due to an alternative etiology (e.g., therapeutic anticoagulation)

2) Liver transplantation or qualification for liver transplantation, defined as MELD score ≥ 15 (unless due to therapeutic anticoagulation) on at least 2 consecutive occasions at least 28 days apart

3) All-cause mortality

Each of the clinical events (except all-cause mortality and liver transplantation) will require confirmation by a Hepatic Events Adjudication Committee. All deaths will be reviewed by this committee to determine if they are liver-related.

Once the clinical event (except all-cause mortality and liver transplantation) is confirmed by the Hepatic Events Adjudication Committee, the subject will be required to discontinue study drug. The subject will complete the Early Termination visit within 5 days of discontinuing treatment and will be followed for clinical outcomes until the Week 96 visit. Follow-up for clinical outcomes may be conducted by phone or in-clinic and the frequency for follow-up may be increased or decreased, at the Investigator's discretion.

Subjects who experience all-cause mortality and liver transplantation will be discontinued from the study, but do not require confirmation from the adjudication committee prior to discontinuation.

6.23.8.2. Cardiovascular Events Adjudication Committee – Main Study Only

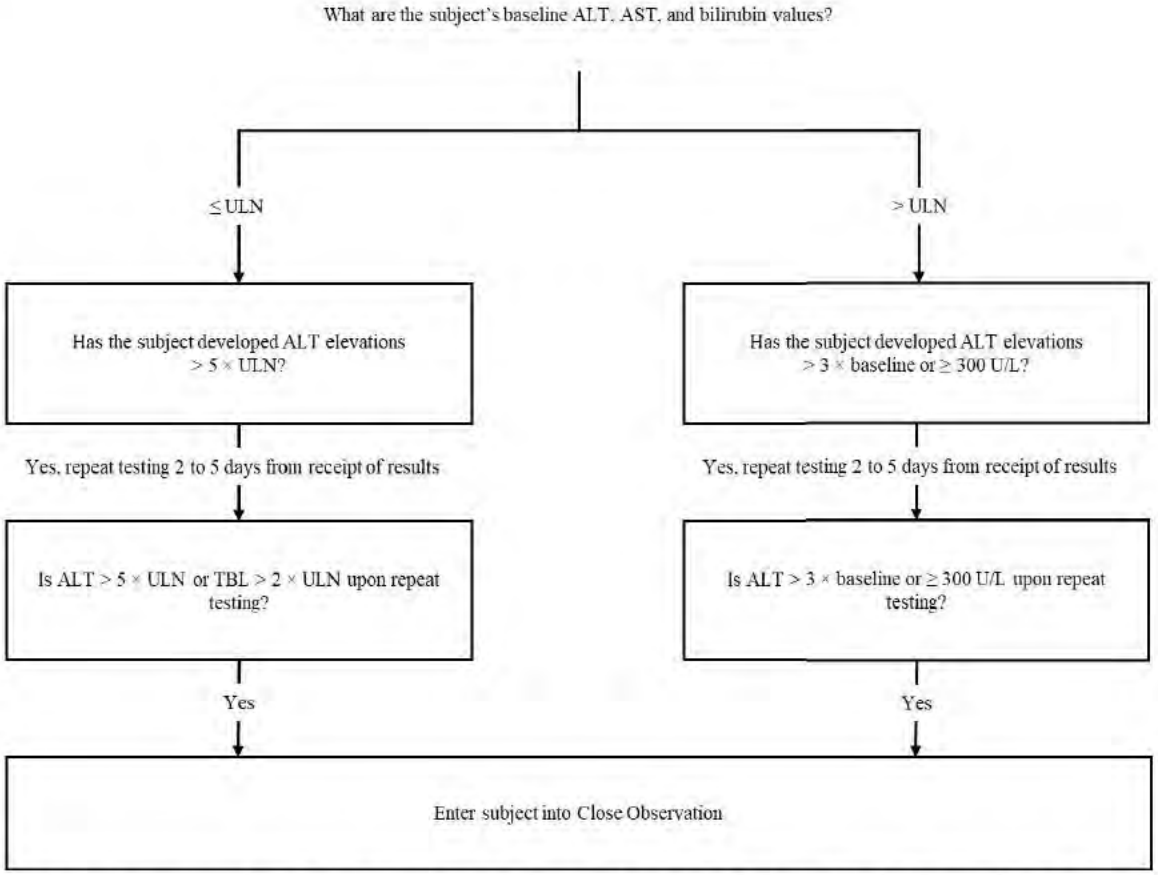
Cardiovascular events including cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, hospitalization for cardiac failure, and urgent or emergency visits for urgent heart failure will be adjudicated by an independent Cardiovascular Events Adjudication Committee. All deaths will be reviewed by this committee to determine if they are cardiovascular-related. Subjects experiencing a cardiovascular event will be allowed to continue in the study at the discretion of the Principal Investigator.

6.23.9. Observation for Drug-Induced Liver Injury (DILI)

Throughout the study, all TEAEs, clinical assessments, and clinical laboratory parameters, and the criteria for potential DILI will be closely monitored with subjects entering close observation as shown in Figure 6-1 and Figure 6-2 and as detailed in Section 6.23.9.1 to Section 6.23.9.3.

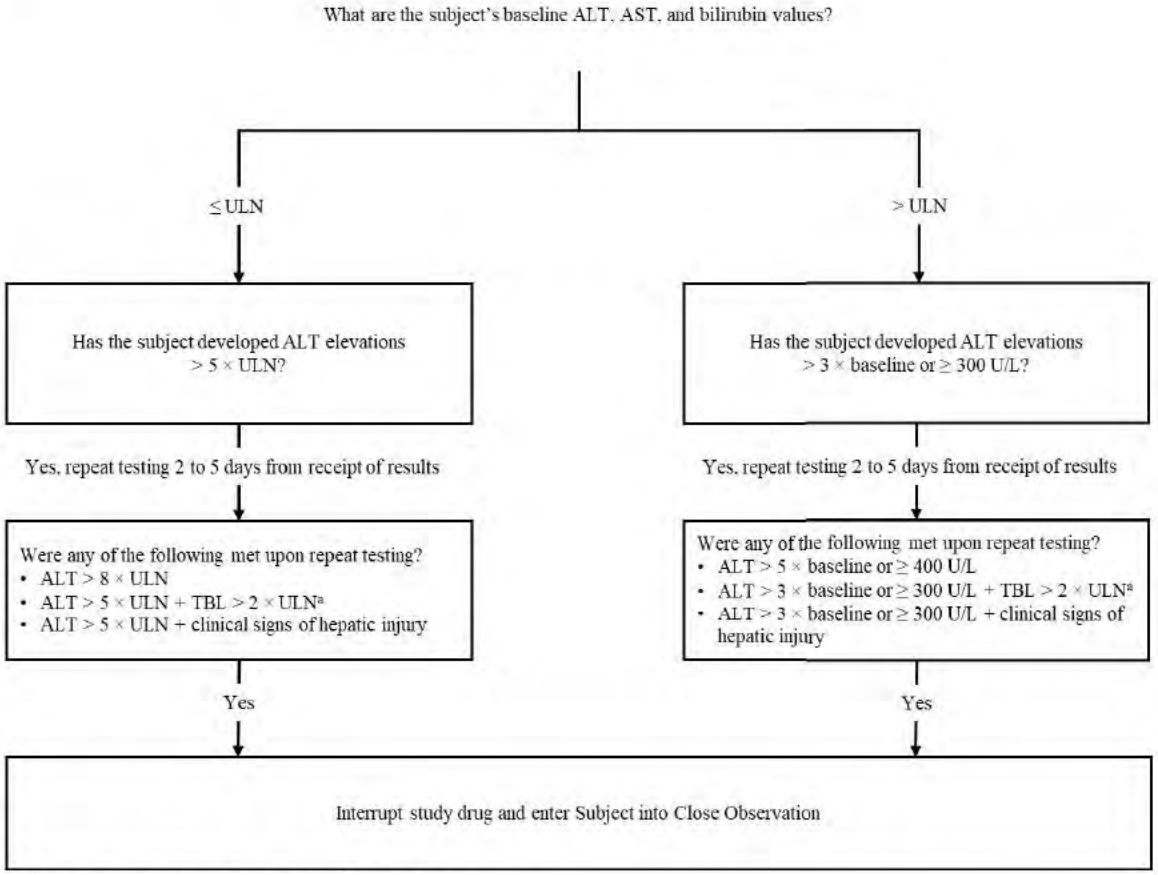
Baseline ALT and AST will be the average of available pre-dose measurements from screening to Day 1.

Figure 6-1. Considerations for Close Observation



ALT = alanine aminotransferase; AST = aspartate aminotransferase; ULN = upper limit of normal, TBL = total bilirubin

Figure 6-2. Considerations for Study Drug Interruption



a Unless the subject has Gilbert's syndrome

ALT = alanine aminotransferase; AST = aspartate aminotransferase; ULN = upper limit of normal, TBL = total bilirubin

6.23.9.1. Subjects with Normal Liver Transaminases and Bilirubin at Baseline

Drug-induced liver injury monitoring in subjects with normal liver transaminases and bilirubin at baseline should be performed throughout the study according to the procedures summarized below.

- If subjects with normal baseline liver indices develop elevations in ALT of > 5 × ULN during the study, repeat testing should be performed within 2 to 5 days from receipt of results

  — If there are persistent elevations (ALT > 5 × ULN or total bilirubin > 2 × ULN) upon repeat testing, close observation (testing and physical examination 2 to 3 times per week) should be implemented. An important purpose of the close observation is to gather additional clinical information to seek other possible causes of the observed liver test abnormalities, such as one of the following: acute viral hepatitis, alcoholic and autoimmune hepatitis, hepatobiliary disorders, cardiovascular causes, or concomitant treatments. Discontinuation of study drug should be considered

- Study drug should be interrupted, and the subject should be followed until resolution of signs or symptoms, in the following situations:

  — ALT > 8 × ULN

  — ALT > 5 × ULN and total bilirubin > 2 × ULN (unless the subject has Gilbert's syndrome)

  — ALT > 5 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (> 5%) as assessed by the Principal Investigator to indicate hepatic injury

For any subjects who present with a constellation of syndromes indicative of liver disease as per the Investigator's overall assessment (i.e., fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia [> 5%]), perform liver function tests to determine if liver disease is worsening.

Re-initiation of study drug may be considered after consultation with the Medical Monitor and DMC assessment.

6.23.9.2. Subjects with Elevations in Liver Transaminases or Bilirubin at Baseline

Drug-induced liver injury monitoring in subjects with elevations in liver transaminases or bilirubin at baseline should be performed throughout the study according to the procedures summarized below.

- If subjects with abnormal baseline liver indices develop elevations of ALT > 3 × baseline or ≥ 300 U/L (whichever occurs first) during the study, repeat testing should be performed within 2 to 5 days from receipt of results

  — If there are persistent elevations (ALT > 3 × baseline, or ≥ 300 U/L) upon repeat testing, then close observation (testing and physical examination 2 to 3 times per week) should be implemented and discontinuation of study drug should be considered

- Study drug should be interrupted, and the subject should be followed until resolution of signs or symptoms, if any of the following occur:

  — ALT increase > 5 × baseline measurements or ≥ 400 U/L (whichever occurs first)

  — ALT increase > 3 × baseline measurements or ≥ 300 U/L (whichever occurs first) AND the increase is accompanied by a concomitant increase in total bilirubin to > 2 × ULN (unless the subject has Gilbert's syndrome)

  — ALT increase > 3 × baseline measurements or ≥ 300 U/L (whichever occurs first) with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (> 5%)

For any subjects who present with a constellation of syndromes indicative of liver disease as per the Investigator's overall assessment (i.e., fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia [> 5%]), perform liver function tests to determine if liver disease is worsening.

Re-initiation of study drug may be considered after consultation with the Medical Monitor and DMC assessment.

6.23.9.3. Close Observation for Suspected Drug-Induced Liver Injury (DILI)

For all subjects, close observation for suspected DILI includes the following within 72 hours of suspected DILI:

- Repeat liver enzyme (ALT, AST, and alkaline phosphatase [ALP]) and serum bilirubin tests 2 or 3 times weekly
- The frequency of repeat testing can decrease to once a week or less if abnormalities stabilize or the study drug has been discontinued and the subject is asymptomatic
- Obtain a more detailed history of symptoms and prior or concurrent diseases
- Obtain 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
- Obtain a history of exposure to environmental chemical agents
- Obtain additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin)
- Consider gastroenterology or hepatology consultations
- Subjects who live far from study sites can be evaluated locally for history, physical exam, and laboratories, provided results can be communicated promptly to the site Investigator

6.23.10. Removal of Subjects from the Study

The Investigator may withdraw a subject from the study for any of the following reasons:

- Withdrawal of consent
- Non-compliance, as determined by the Investigator or Sponsor, including refusal to complete the Week 36 liver biopsy
- At the discretion of the Investigator and/or Medical Monitor and/or the DMC, if it is in the best interest of the subject

- Lost to follow-up

At any time, the Investigator can remove a subject from study treatment and/or the study if deemed necessary for subject safety. The Sponsor also reserves the right to terminate the study at any time. All data normally collected at completion of the study must be collected at the time of the subject's early termination or termination of the study.

The Investigator will discuss with the subject or legally acceptable representative(s) the most appropriate way to withdraw to ensure the subject's health. Should a subject (or a legally acceptable representative) request or decide to withdraw consent, all efforts will be made to complete and report the observations as thoroughly as possible up to the date of withdrawal.

7. PLANNED STATISTICAL METHODS

7.1. Endpoints

Details will be provided in the statistical analysis plan (SAP).

7.1.1. Efficacy Endpoints

7.1.1.1. Primary Endpoint

For a complete list of endpoints, refer to Section 2.

7.1.1.2. Secondary Endpoints

For a complete list of endpoints, refer to Section 2.

7.1.1.3. Exploratory Efficacy Endpoints

For a complete list of endpoints, refer to Section 2.

7.1.2. Safety Endpoints

Main Study: The safety endpoint is safety and tolerability of EFX in subjects with NASH.

Cohort D: The safety endpoint is the safety and tolerability of EFX in subjects with T2D and liver fibrosis due to NASH when added to an existing GLP-1 receptor agonist.

Safety analyses may include: summaries of extent of exposure, AEs, clinical laboratory tests, ECGs, ultrasounds (**main study** only), bone mineral density (BMD; **main study** only), vital sign assessments, body weight, ADA and NAb assessments, and concomitant medication usage.

7.2. Planned Analyses

7.2.1. Primary Endpoint Analysis – Main Study Only

A Week 36 primary endpoint analysis is planned after all randomized subjects have completed their Week 36 visit (or prematurely discontinued from the study prior to Week 36). The analysis may be submitted to regulatory agencies to seek guidance for the overall clinical development program.

At the database lock for the Primary Analysis, limited personnel from Sponsor and its designee (e.g., project statistical team who will be analyzing the data) will be unblinded to the treatment allocation. All other Sponsor personnel and its designee, Investigators, study site personnel, and study participants will remain blinded to the treatment allocation until the final database lock. Details regarding the management of unblinded data will be described in the SAP and study Blinding Plan.

7.2.2. Final Analysis

The final analysis for the **main study** and **Cohort D** will be performed after all subjects have completed the respective study, outstanding data queries have been resolved or adjudicated as unresolvable, and the data have been cleaned and finalized.

7.3. Analysis Conventions

All individual subject data will be listed as measured. All statistical summaries and analyses will be performed using Statistical Analysis System® (SAS®) software (SAS Institute, Cary, North Carolina, USA).

7.4. Analysis Sets

The study analysis sets are detailed below.

7.4.1. Full Analysis Set (FAS)

The FAS will include all subjects who were randomized and received at least one dose of study drug.

Subjects who receive a dose of study drug other than that to which they were randomized for the entire duration of treatment will be analyzed according to treatment group to which they were randomized.

7.4.2. Per Protocol (PP) Sets

The Per Protocol (PP) Sets will be a subset of the FAS and will include subjects who finish scheduled visits with applicable assessments and do not have major protocol deviations.

7.4.3. Safety Analysis Set

All subjects who receive at least 1 dose of study drug will be included in the Safety Analysis Set. All safety endpoints will be summarized using the Safety Analysis Set and will be based on actual treatment received if this differs from the randomized treatment.

7.4.4. Pharmacokinetics Set

The PK analysis set will include all randomized subjects who took at least one dose of study drug and for whom concentration data for EFX is available. Subjects in this analysis set will be summarized according to the actual treatment received.

7.4.5. Biomarkers Analysis Set

The Biomarker Analysis Set will include data from subjects in the Safety Analysis Set who have the necessary baseline and on-study measurements to provide interpretable results for the specific parameters of interest.

7.4.6. Liver Biopsy Analysis Set (LBAS) – Main Study Only

The analysis set for histological efficacy analyses will be the Liver Biopsy Analysis Set (LBAS), which will be a subset of the FAS. It will include all FAS subjects who have the baseline and on-study liver biopsy results.

7.4.7. MRI-PDFF Analysis Set – Cohort D Only

The MRI-PDFF Analysis Set will be a subset of the FAS. It will include all FAS subjects who have baseline and on-study measurements assessed by MRI-PDFF.

7.5. Data Handling Conventions

Missing data can have an impact on the interpretation of the trial data. In general, values for missing data will not be imputed unless specified.

Where appropriate, safety data for subjects who did not complete the study will be included in summary statistics. For example, if a subject received study drug, the subject will be included in a summary of AEs according to the treatment received; otherwise, if the subject was not dosed then the subject will be excluded from the summary. If safety laboratory results for a subject are missing for any reason at a time point, the subject will be excluded from the calculation of summary statistics for that time point. If the subject is missing a baseline value, then the subject will be excluded from the calculation of summary statistics for the baseline value and the change from baseline values.

Values for missing safety laboratory data will not be imputed; however, a missing baseline result will be replaced with a screening result, if available. If no pre-treatment laboratory value is available, the baseline value will be assumed to be normal (i.e., no grade [Grade 0]) for the summary of graded laboratory abnormalities.

Values for missing vital signs data will not be imputed; however, a missing baseline result will be replaced with a screening result, if available.

Further details of data handling conventions and transformation will be provided in the SAP.

7.6. Estimands – Main Study Only

In line with International Council for Harmonisation (ICH) E9 (R1) addendum, 5 attributes (treatment, population, endpoint, intercurrent events, and population-level summary) have been specified to translate the primary and key secondary efficacy objectives into treatment effects that are to be estimated (estimands).

The treatment conditions of interest are EFX 28 mg administered weekly, and EFX 50 mg administered weekly, and are compared with placebo administered weekly.

The treatment effect is assessed by the primary histological endpoint of improvement in liver fibrosis and no worsening of steatohepatitis at Week 36.

The population of subjects targeted is adults with compensated cirrhosis due to NASH as defined by the inclusion/exclusion criteria.

Intercurrent events are handled through a composite strategy (see Table 12).

The population-level summary of the treatment effect will be quantified by comparing observed responder proportions supported by the risk ratio of EFX compared to placebo (EFX 28 mg versus placebo or EFX 50 mg versus placebo) calculated from the odds ratio obtained through Cochran-Mantel-Haenszel (CMH) test detailed in the SAP.

Table 12. Approach for Intercurrent Events for the Primary Endpoint

Intercurrent Event	Status
Liver Transplant	Subject defined as a non-responder (even if a post-treatment biopsy is available)
MELD score ≥ 15	
Hepatic decompensation event	
Bariatric surgery (e.g. gastroplasty, Roux-en-Y gastric bypass) performed prior to biopsy at Week 36	Subject defined as a non-responder

MELD = Model for End-Stage Liver Disease

The estimands attributes for all secondary histological endpoints are defined in the same manner as for the primary endpoint described above.

7.7. Demographic Data and Baseline Characteristics

Demographic and baseline measurements will be summarized using standard descriptive methods.

Demographic summaries will include T2D status, sex, race/ethnicity, and age.

Baseline data will include a summary of body weight, height, BMI, randomization stratification groups, and other disease characteristic variables.

7.8. Efficacy Analysis

7.8.1. Primary Efficacy Analysis – Main Study Only

The primary efficacy analysis will be conducted when all of the planned subjects complete the Week 36 visit. A CMH test will be used to compare the differences in proportions of subjects who achieve a ≥ 1 stage improvement in fibrosis without worsening of steatohepatitis at Week 36 between the EFX arms and the placebo arm, adjusting for stratification factors. The analysis will be performed using the LBAS. The point estimates and 95% confidence intervals for the differences in proportions will be calculated.

The primary efficacy endpoint will be tested at Type I error rate of 0.05, two-sided without adjustment for multiplicity.

The primary endpoint analysis will be repeated in the FAS where subjects with missing biopsies will be considered non-responders.

7.8.2. Secondary Efficacy Analysis – Main Study Only

For the secondary endpoints, a CMH test will be performed to compare the differences in proportions between the EFX arm and the placebo arm adjusting for stratification factors.

An analysis of covariance (ANCOVA) model with baseline as a covariate adjusting for stratification factors will be used to evaluate the changes from baseline to Week 36 in liver stiffness between either EFX arm and the placebo arm in the Week 36 analysis.

A mixed-model repeated-measures (MMRM) with baseline as a covariate will be used to evaluate the changes from baseline in ELF score, Pro-C3, TG, total cholesterol, HDL-C, non-HDL—C, LDL-C, HbA1c, C-peptide, adiponectin, insulin, HOMA-IR, non-invasive fibrosis markers, liver stiffness, and body weight between the EFX arm and the placebo arm at scheduled visits, adjusting for stratification factors.

Summaries and will be presented for changes from baseline in ELF score, Pro-C3, TG, total cholesterol, HDL-C, non-HDL—C, LDL-C, HbA1c, C-peptide, insulin, HOMA-IR, and body weight at the collection time points defined in the Schedule of Assessments (Appendix A and Appendix B) by treatment arm.

7.8.3. Exploratory Efficacy Analysis – Main Study Only

[redacted]

7.8.4. Cohort D Efficacy Analyses

Summary statistics (number of subjects, mean, standard deviation, median, minimum, and maximum) at all visits and change from baseline will be provided. The continuous efficacy variables will be analyzed with an ANCOVA model or MMRM with treatment group and liver fibrosis stage (1 vs 2-3) as factors, and baseline value as a covariate. Normality will be tested for the model residuals.

7.9. Safety Analysis

All safety data collected on or after the date that study drug is first dispensed up to the date of last dose of study drugs plus 30 days will be summarized by treatment group. Data for the follow-up period will be included in data listings.

7.9.1. Extent of Exposure

Data of a subject's extent of exposure to study drug will be generated from the data recorded in the study drug administration eCRF. Exposure data will be summarized by treatment group.

7.9.2. Adverse Events

Clinical and laboratory AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). System Organ Class (SOC), High-Level Group Term (HLGT), High-Level Term (HLT), PT, and Lower-Level Term (LLT) will be attached to the clinical database. Adverse event severity will be graded using the CTCAE v5.0.

Events will be summarized on the basis of the date of onset for the event. A TEAE will be defined as one or both of the following:

- Any AEs with an onset date on or after the study drug start date and no later than 30 days after permanent discontinuation of study drug
- Any AEs leading to premature discontinuation of study drug

Summaries (number and percentage of subjects) of TEAEs and SAEs by SOC and PT will be provided by treatment group. Treatment-emergent AEs will also be summarized by relationship to study drugs and severity. In addition, TEAEs leading to premature discontinuation of study drugs and study, and SAEs leading to death will be summarized and listed.

All AEs collected during the course of the study will be presented in data listings with a field for treatment-emergent event (Yes/No).

7.9.3. Laboratory Evaluations

Selected laboratory data will be summarized (n, mean, standard deviation, median, minimum, and maximum) by treatment group and study visit along with the corresponding change from baseline values.

Graded laboratory abnormalities will be defined using the grading scheme in the CTCAE v5.0. Grading of laboratory abnormalities for analysis purposes will be defined in the SAP.

The incidence of treatment-emergent laboratory abnormalities, defined as values that increase at least one toxicity grade from baseline at any time post-baseline up to and including the date of last dose of study drugs plus 30 days, will be summarized by treatment group. If baseline data are missing, then any graded abnormality (i.e., at least a Grade 1) will be considered treatment-emergent.

7.10. Pharmacokinetic Analysis

Plasma concentrations of EFX will be listed and summarized pre-dose at the time points listed in the Schedule of Assessments (Appendix A, Appendix B, and Appendix C). Noncompartmental parameters will be listed and summarized descriptively.

7.11. Biomarker Analysis

Descriptive statistics of biomarker change from baseline will be provided at each sampling time by treatment. Point estimates and 95% confidence intervals may be calculated.

[redacted]

7.12. Immunogenicity Analysis

The incidence of pre-existing, treatment-emergent positive ADA, positive ADA at any time point, negative ADA at all time points, and NAB, if any, will be summarized by treatment and period. Pre-existing ADA is defined as positive ADA result at or before dosing. Treatment-emergent positive ADA is defined as negative result at or before dosing and positive result post-dose. Titers will also be summarized descriptively for positive ADAs.

Efficacy and safety variables may be analyzed based on the positive ADA and negative ADA subjects. Additionally, the impact of immunogenicity on PK and pharmacodynamics will be analyzed.

7.13. Sample Size

The primary endpoint for the **main study** is the proportion of subjects who achieve ≥ 1 stage improvement in fibrosis and no worsening of steatohepatitis at Week 36. Based on results of published studies in NASH with compensated cirrhosis and results of Cohort C in the Phase 2a BALANCED study of EFX, it is estimated that approximately 12% of subjects on placebo and 42% of subjects on EFX will reach this endpoint.

With a two-sided CMH test for proportion difference, at a significance level of 0.05, 45 completed subjects per group will provide at least 90% power. Sample size calculations are for testing 2 proportions using a CMH test and were computed using the normal approximation method. Factoring in dropouts, the study plans to randomize at least 150 subjects in total (50 subjects per group). The main study planned to enroll 150 subjects, but over enrolled by approximately 20%.

Since the primary objective of **Cohort D** is to assess the safety and tolerability of EFX compared to placebo when added to an existing GLP-1 receptor agonist in subjects with T2D and liver fibrosis due to NASH, the sample size is determined based on clinical considerations. The study is not powered for comparisons of efficacy measurements across treatment groups.

7.14. Data Monitoring Committee

An external DMC that consists of at least 1 hepatologist, 1 endocrinologist, 1 cardiologist, and 1 statistician will review the progress of the study. The DMC will convene after 50% of subjects in

the **main study** have received at least 1 dose of study drug and have either completed the Week 12 assessments or prematurely discontinued. The DMC will review any available **Cohort D** subject data at that time. The DMC will receive all reports of SAEs, potential DILI events for evaluation and convene as needed to monitor for safety. Additional meetings will be scheduled, as required. The DMC will provide recommendations to the Sponsor whether the nature, frequency, and severity of adverse effects associated with study treatment warrant the early termination of the study in the best interests of the participants, whether the study should continue as planned, or the study should continue with modifications. The DMC may also provide recommendations, as needed, regarding study design.

The DMC will monitor study progress; tracking the actual annual event rate for the outcomes endpoint.

Due to the challenge of recognizing and diagnosing DILI in subjects with pre-existing hepatic dysfunction, the DMC will review potential cases of DILI (refer to Section 6.23.9). Subjects will be categorized as those for whom DILI or worsening of hepatic function attributable to study drug could be excluded (e.g., a clear, alternative explanation exists); those for whom DILI or worsening of hepatic function attributable to study drug could not be excluded (e.g., no clear, alternative explanation exists); and those with insufficient data to make a determination. Subjects experiencing DILI may be allowed to continue in the study according to the follow-up procedures outlined in Section 6.23.9.

The DMC's specific activities will be defined by a mutually agreed charter, which will define the DMC's membership, conduct, and meeting schedule.

While the DMC will be asked to advise the Sponsor regarding future conduct of the study, including possible early study termination, the Sponsor retains final decision-making authority on all aspects of the study.

8. ADMINISTRATIVE CONSIDERATIONS

8.1. Investigator Responsibilities

8.1.1. Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki, ICH guidelines, or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the study subject. These standards are consistent with the EU Clinical Trials Directive 2001/20/EC and GCP Directive 2005/28/EC.

The Investigator will ensure adherence to the basic principles of GCP, as outlined in 21 CFR 312, subpart D, "Responsibilities of Sponsors and Investigators," 21 CFR, Part 50, 1998, and 21 CFR, Part 56, 1998.

The Investigator and all applicable sub-Investigators will comply with 21 CFR, Part 54, 1998, providing documentation of their financial interest or arrangements with the Sponsor, or proprietary interests in the study drug under study. This documentation must be provided prior to the Investigator's (and any sub-Investigator's) participation in the study. The Investigator and sub-Investigator agree to notify Akero of any change in reportable interests during the study and for 1 year following completion of the study. Study completion is defined as the date when the last subject completes the protocol-defined activities.

8.1.2. Institutional Review Board (IRB)/Independent Ethics Committee (IEC) Review and Approval

Before initiating a trial, the Investigator (or Sponsor, as appropriate according to local regulations) will submit this protocol, ICF, and any accompanying material to be provided to the subject (such as advertisements, subject information sheets, or descriptions of the study used to obtain informed consent) to an IRB/IEC. The Investigator will not begin any study subject activities until approval from the IRB/IEC has been documented and provided as a letter to the Investigator.

Before implementation, the Investigator will submit to and receive documented approval from the IRB/IEC any modifications made to the protocol or any accompanying material to be provided to the subject after initial IRB/IEC approval, with the exception of those necessary to reduce immediate risk to study subjects.

8.1.3. Informed Consent

Eligible subjects may only be included in the study after providing (witnessed, where required by law or regulation), IRB/IEC-approved informed consent, or, if applicable after such consent has been provided by a legally acceptable representative(s) of the subject. Informed consent must be obtained before conducting any study-specific procedures (e.g., all of the procedures described in

the protocol). The process of obtaining informed consent must be documented in the subject source documents.

The Investigator must use the most current IRB/IEC-approved consent form for documenting written informed consent.

8.1.4. Confidentiality

The Investigator will ensure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only partial date of birth (as applicable in certain countries), another unique identifier (as allowed by local law), and an identification code will be recorded on any form or biological sample submitted to the Sponsor, IRB/IEC, or laboratory. Laboratory specimens must be labeled in such a way as to protect subject identity while allowing the results to be recorded to the proper subject. Refer to specific laboratory instructions. The Investigator will keep a screening log showing study subject IDs, names, and addresses for all subjects screened and for all subjects randomized in the trial. Subject data will be processed in accordance with all applicable regulations.

The Investigator agrees that all information received from Akero, including but not limited to the IB, this protocol, eCRFs, the study drug, and any other study information, remain the sole and exclusive property of Akero during the conduct of the study and thereafter. This information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the study or as required by law) without prior written consent from Akero. The Investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study site to any third party or otherwise into the public domain.

8.1.5. Study Files and Retention of Records

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into at least the following 2 categories: (1) Investigator's study file, and (2) subject clinical source documents.

The Investigator's study file will contain the protocol/amendments, case report forms (CRF) and query forms, IRB/IEC and governmental approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and correspondence.

The Investigator must maintain source documents for each subject in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, ECGs, and the results of any other tests or assessments. All information on CRFs must be traceable to these source documents in the subject's file. Data not requiring a separate written record will be defined before study start and will be recorded directly on the CRFs. The Investigator must also keep the original ICF signed by the subject (a signed copy is given to the subject).

The Investigator must give the monitor access to all relevant source documents to confirm their consistency with the data capture and/or data entry. Akero monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs, and of data that will be used for all primary variables. Additional checks of the consistency of the source data with the CRFs are performed according to the study-specific monitoring plan. No information in source documents about the identity of the subjects will be disclosed.

All clinical study documents must be retained by the Investigator until at least 2 years or for a period according to local laws, whichever is longer, after the last approval of a marketing application in an ICH region (i.e., US, Europe, or Japan) and until there are no pending or planned marketing applications in an ICH region; or, if no application is filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and regulatory authorities have been notified. Investigators may be required to retain documents longer if specified by regulatory requirements, by local regulations, or by an agreement with Akero. The Investigator must notify Akero before destroying any clinical study records.

Should the Investigator wish to assign the study records to another party or move them to another location, Akero must be notified in advance.

If the Investigator cannot provide for this archiving requirement at the study site for any or all of the documents, special arrangements must be made between the Investigator and Akero to store these records securely away from the site so that they can be returned sealed to the Investigator in case of an inspection. When source documents are required for the continued care of the subject, appropriate copies should be made for storage away from the site.

8.1.6. Electronic Case Report Forms

Designated Investigator-staff will enter the data required by the protocol into the eCRF using fully validated secure web-enabled software that conforms to US CFR 21 Part 11 requirements. Designated Investigator site staff will not be given access to the system until they have been trained. Subsequent to data entry, a study monitor will perform source data verification within the EDC system. Original entries as well as any changes to data fields will be stored in the audit trail of the system.

Automatic validation procedures within the system check for data discrepancies during and after data entry and, by generating appropriate error messages, allow the data to be confirmed or corrected online by the designated Investigator-site staff. The Investigator must certify that the data entered into the eCRF are complete and accurate.

At the conclusion of the trial, Akero will provide the site with a read-only archive copy of the data entered by that site. This archive must be stored in accordance with the records retention requirements outlined in Section 8.1.5.

8.1.7. Inspections

The Investigator will make available all source documents and other records for this trial to Akero's appointed study monitors, to IRB/IEC, or to regulatory authority or health authority inspectors.

8.1.8. Protocol Compliance

The Investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol.

8.2. Sponsor Responsibilities

8.2.1. Protocol Modifications

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made only by Akero. The Investigator must submit all protocol modifications to the IRB/IEC in accordance with local requirements and receive documented IRB/IEC approval before modifications can be implemented.

8.2.2. Study Report and Publications

A clinical study report (CSR) will be prepared and provided to the regulatory agencies. Akero will ensure that the report meets the standards set out in the ICH Guideline for Structure and Content of Clinical Study Reports (ICH E3). Note that an abbreviated report may be prepared in certain cases.

Investigators in this study may communicate, orally present, or publish in scientific journals or other scholarly media only after the following conditions have been met: the results of the study in their entirety have been publicly disclosed by, or with the consent of, Akero in an abstract, manuscript, or presentation form or the study has been completed at all study sites for at least 18 months.

The Investigator will submit to Akero any proposed publication or presentation along with the respective scientific journal or presentation forum at least 60 days before submission of the publication or presentation.

No such communication, presentation, or publication will include Akero's confidential information (see Section 8.1.4).

The Investigator will comply with Akero's request to delete references to its confidential information (other than the study results) in any paper or presentation and agrees to withhold publication or presentation for an additional 60 days in order to obtain patent protection if deemed necessary.

8.3. Joint Investigator/Sponsor Responsibilities

8.3.1. Payment Reporting

Investigators and their study staff may be asked to provide services performed under this protocol (e.g., attendance at Investigator's Meetings). If required under the applicable statutory and regulatory requirements, Akero will capture and disclose to Federal and State agencies any expenses paid or reimbursed for such services, including any clinical trial payments, meal, travel expenses or reimbursements, consulting fees, and any other transfer of value.

8.3.2. Access to Information for Auditing or Inspections

Representatives of regulatory authorities or of Akero may conduct inspections or audits of the clinical study. If the Investigator is notified of an inspection by a regulatory authority, the Investigator agrees to notify the Medical Monitor immediately. The Investigator agrees to provide to representatives of a regulatory agency or Akero access to records, facilities, and personnel for the effective conduct of any inspection or audit.

8.3.3. Study Discontinuation

Both the Sponsor and the Investigator reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange discontinuation procedures and notify the subjects, appropriate regulatory authorities, and IRBs/IECs. In terminating the study, Akero and the Investigator will assure that adequate consideration is given to the protection of the subjects' interests.

9. REFERENCE LIST

Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver fibrosis, but no other histologic features, is associate with long-term outcomes in patients with nonalcoholic fatty liver disease. Gastroenterology. 2015; 149:389-397.

Arnett D.K., Blumenthal R.S., Albert M.A., Buroker A.B., et al., 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines, J. Am. Heart Assoc., Circulation. 2019;140:e596–e646.

Bao L, Yin J, Gao W, et al. A long-acting FGF21 alleviates hepatic steatosis and inflammation in a mouse model of non-alcoholic steatohepatitis partly through an FGF21-adiponectin-IL17A pathway. Br J Pharmacol. 2018; 175: 3379-3393.

Camporez JP, Jornayvaz FR, Petersen MC, et al. Cellular mechanisms by which FGF21 improves insulin sensitivity in male mice. Endocrinology. 2013; 154: 3099–3109.

Chalasani N, Younossi Z, Lavine JF, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the study of liver diseases. Hepatology. 2018; 67(1):328-357.

Chen MS, Chang JC, Zavala-Solorio J, et al. FGF21 mimetic antibody stimulates UCP1-independent brown fat thermogenesis via FGFR1/bKlotho complex in non-adipocytes. Mol. Metab. 2017; 6: 1454-1467.

Coskun T, Bina HA, Schneider MA, et al. Fibroblast growth factor 21 corrects obesity in mice. Endocrinology. 2008; 149: 6018–6027.

Cui A, Li J, Ji S, Ma F, Wang G, Xue Y, et al. The effects of B1344, a novel fibroblast growth factor 21 analog, on nonalcoholic steatohepatitis in nonhuman primates. Diabetes. 2020.

Dulai PS, Singh S, Patel J, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systemic review and meta-analysis. Hepatology. 2017; 65(5):1557-1565.

Ekstedt M, Hagström H, Nasr P, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology. 2015; 61: 1547-54.

Estes C, Anstee QM, Arias-Loste MT et al. Modeling NAFLD disease burden in China, France,Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. J. Hepatol. 2018; 69;896–904.

European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical practice guidelines for the management of non-alcoholic fatty liver disease. J. Hepatol, 2016; 64(6):1388-1402.

Fisher FM, Chui PC, Nasser IA, et al. Fibroblast growth factor 21 limits lipotoxicity by promoting hepatic fatty acid activation in mice on methionine and choline-deficient diets. Gastroenterology. 2014; 147: 1073-83.

Fon Tacer K, Bookout AL, Ding X, et al. Research resource: Comprehensive expression atlas of the fibroblast growth factor system in adult mouse. Mol Endocrinol. 2010; 24: 2050-2064.

Gaich G, Chien JY, Fu H, et al. The effects of LY2405319, and FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metab. 2013; 18(3):333-340.

Gimeno RE, Moller DE. FGF21-based pharmacotherapy: Potential utility for metabolic disorders. Trends Endocrinol. Metabol. 2014; 25: 303-311.

Hagström H, Nasr P, Esktedt M, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J. Hepatology. 2017; 67:1265-1273.

Kaufman A, AbuqayyasL, Denney WS, Tillman EJ, Rolph T. AKR-001, and Fc-FGF21 analog, showed sustained pharmacodynamic effects on insulin sensitivity and lipid metabolism in type 2 diabetes patients. Cell Reports Medicine. 2020; 1:100057.

Kim AM, Somayaji VR, Dong JQ, et al. Once-weekly administration of a long-acting fibroblast growth factor 21 analogue modulates lipids, bone turnover markers, blood pressure and body weight differently in obese people with hypertriglyceridaemia and in non-human primates. Diabetes Obes Metab. 2017; 19(12):1762-1772.

Lee JH, Kang YE, Chang JY, et al. An engineered FGF21 variant, LY2405319, can prevent non-alcoholic steatohepatitis by enhancing hepatic mitochondrial function. Am J Transl Res. 2016; 8: 4750-4763.

Li H, Zhang J, Jia W. Fibroblast growth factor 21: a novel metabolic regulator from pharmacology to physiology. Front Med. 2013; 7: 25–30.

Lin Z, Tian H, Lam KS, et al. Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. Cell Metab. 2013; 17: 779–789.

Mu J, Pinkstaff J, Li Z, et al. FGF21 analogs of sustained action enabled by orthogonal biosynthesis demonstrate enhanced antidiabetic pharmacology in rodents. Diabetes. 2012; 61: 505–512.

Nishimura T, Nakatake Y, Konishi M, Itoh N. Identification of a novel FGF, FGF-21, preferentially expressed in the liver. Biochim Biophys Acta. 2000; 1492: 203-206.

Opoku Y.K, Liu Z, Afrifa J, Kumi A.K, Liu H, Ghartey-Kwansah G, Koranteng H, et al. Fibroblast Growth Factor-21 Ameliorates Hepatic Encephalopathy by Activating the STAT3-SOCS2 Pathway to Inhibit Activated Hepatic Stellate Cells. EXCLI: 19:567-581, 2020.

Ruhl CE, Everhart JE. Fatty liver indices in the multiethnic United States National Health and Nutrition Examination Survey. Aliment Pharmacol. Ther. 2015; 41: 65–76.

Stanislaus S, Hecht R, Yie J, Hager T, Hall M, Spahr C, et al. A Novel Fc-FGF21 With Improved Resistance to Proteolysis, Increased Affinity Toward b-Klotho, and Enhanced Efficacy in Mice and Cynomolgus Monkeys. Endocrinology 158: 1314–1327, 2017.

Talukdar S, Zhou Y, Li D, et al. A long-acting FGF21 molecule, PF-05231023, decreases body weight and improves lipid profile in non-human primates and type 2 diabetic subjects. Cell Metab. 2016; 23: 427-440.

Tanaka N, Takahashi S, Zhang Y, et al. Role of fibroblast growth factor 21 in the early stage of NASH induced by methionine- and choline-deficient diet. Biochim. Biophysica. Acta. 2015; 1852: 1242-1252.

The NASH Education Program. https://www.the-nash-education-program.com/. Accessed February 25, 2019.

Targher G, Byrne C.D, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease and risk incident cardiovascular disease: A meta-analysis. Journal of Hepatology. Vol 65: 589-600. 2016.

Wu G, Li H, Fang Q, et al. Complementary role of fibroblast growth factor 21 and cytokeratin 18 in monitoring the different stages of nonalcoholic fatty liver disease. Sci. Rep. 2017; 7: 5095.

Xu J, Lloyd DJ, Hale C, et al. Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. Diabetes. 2009; 58: 250–259.

Xu J, Zhang Y, Liu Y, et al. Fibroblast growth factor 21 attenuates hepatic fibrogenesis through TGF-β/smad2/3 and NF-κB signaling pathways. Toxicol Appl. Pharmacol. 2016; 290: 43-53.

Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease- meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016; 64: 73–84.

Zhao L, Niu J, Lin H, Zhao J, Liu Y, Song Z, Xiang C, et al. Paracrine-endocrine FGF chimeras as potent therapeutics for metabolic diseases. EbioMedicine. 2019; 48: 462-477.

10. APPENDICES

Appendix A. Main Study Schedule of Assessments Table – Screening through Week 36
Appendix B. Main Study Schedule of Assessments Table – Long-Term Follow-up
Appendix C. Cohort D Schedule of Assessments Table
Appendix D. Pregnancy Precautions, Definition for Female of Childbearing Potential, and Contraceptive Requirements
Appendix E. West Haven Criteria
Appendix F. Child-Pugh Score
Appendix G. Definitions of Compensated Cirrhosis Due to Nonalcoholic Steatohepatitis (NASH)

Appendix A. Main Study Schedule of Assessments Table – Screening through Week 36

Visit	Screening[a]	Pre-baseline[b]	Day 1	Week 1	2, 3	4	8	12	16	20	24	28	32	5, 6, 7, 9, 10, 11, 13, 14, 15, 17, 18, 19, 21, 22, 23, 25, 26, 27, 29, 30, 31, 33, 34, 35[c]	36	ET[d]	30-day Follow-up[e]
Visit Window				±3 days												≤ 5 days	±3 days
Written Informed Consent	X																
Eligibility	X	X	X														
Medical History	X																
Complete Physical Examination	X		X					X							X	X	
Symptom-driven Physical Examination				X		X	X		X	X	X	X	X				X
Child-Pugh Score[f]	X		X					X							X	X	X
MELD Score	X		X	X		X	X	X	X	X	X	X	X		X	X	X
Vital Signs[g]	X	X		X	X	X	X		X	X	X	X	X				X
Temperature	X		X														
Triplicate Vital Signs[h]			X					X							X	X	
Height	X																
Weight[i]	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
12-lead ECG	X		X												X	X	X
Abdominal Ultrasound[j]	X										X					X	
FibroScan®[k]	X[l]										X				X	X	
Dual-energy X-ray Absorptiometry	X														X[m]	X[n]	
Liver Biopsy	X[o]														X[p]	X[n]	
Optional MRE Substudy[q]	X[r]														X[s]		
CLDQ-NASH[t]			X					X			X				X	X	
WPAI-NASH[t]			X					X			X				X	X	

Visit	Screening[a]	Pre-baseline[b]	Day	Week											ET[d]	30-day Follow-up[e]
			1	1	2, 3	4	8	12	16	20	24	28	32	5, 6, 7, 9, 10, 11, 13, 14, 15, 17, 18, 19, 21, 22, 23, 25, 26, 27, 29, 30, 31, 33, 34, 35[c]	36		
Visit Window				±3 days												≤ 5 days	±3 days
Lifestyle Guidance[u]	X		X	X	X	X	X	X	X	X	X	X	X		X		
Chemistry[v,w]	X		X	X		X	X	X	X	X	X	X	X		X	X	X
ALT/AST		X															
Hematology[v]	X		X	X		X	X	X	X	X	X	X	X		X	X	X
Coagulation[v]	X		X	X		X	X	X	X	X	X	X	X		X	X	X
Lipoproteins[v,w]	X		X	X		X	X	X	X	X	X	X	X		X	X	X
Serum Storage Samples[v]	X		X	X		X	X	X	X	X	X	X	X		X	X	X
Plasma Storage Samples[v]	X		X	X		X	X	X	X	X	X	X	X		X	X	X
Urinalysis[v]			X					X			X				X	X	
Urine Drug Screen[v]	X																
Serology[v]	X																
Fibrosis Biomarkers[v]	X[x]		X					X			X				X	X	X
Other Biomarkers[v,w]			X			X		X			X				X	X	X
PEth Test[v]			X								X					X	
EFX PK[v,y]			X			X	X		X		X				X	X	X
Optional PK Substudy[v,z]																	
ADA and NAb[v,aa]			X				X		X		X		X		X	X	X
Endogenous FGF21[v,w]			X				X		X		X		X		X	X	X
Optional Genetic Substudy Sample[v,bb]			X														
Optional PBMC Substudy[v,cc]			X			X				X					X	X	X
AAT Test[v]	X																
Ceruloplasmin Test[v]	X																
24-hour Urine Creatinine and Urinary Free Cortisol Tests[v]	X[dd]						X										

Visit	Screening[a]	Pre-baseline[b]	Day 1	Week 1	2, 3	4	8	12	16	20	24	28	32	5, 6, 7, 9, 10, 11, 13, 14, 15, 17, 18, 19, 21, 22, 23, 25, 26, 27, 29, 30, 31, 33, 34, 35[c]	36	ET[d]	30-day Follow-up[e]
Visit Window				±3 days												≤ 5 days	±3 days
Salivary Cortisol Test[v,ee]			X				X		X		X		X			X	X
IGF-1, Free T4, and TSH Tests[v]			X					X			X				X	X	X
Pregnancy Test[v,ff]	X		X			X	X	X	X	X	X	X	X		X	X	X
Adverse Events[gg]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer Study Drug[hh]			X	X	X	X	X	X	X	X	X	X	X	X	X		

AAT = alpha-1-antitrypsin; ADA = anti-drug antibody; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CLDQ-NASH = Chronic Liver Disease Questionnaire-Nonalcoholic Steatohepatitis; ECG = electrocardiogram; EFX = Efruxifermin; FGF21 = fibroblast growth factor 21; IGF-1 = insulin-like growth factor-1; MELD = Model for End-Stage Liver Disease; MRE = magnetic resonance elastography; NAb = neutralizing antibody; PBMC = peripheral blood mononuclear cell; PEth = phosphatidylethanol; PK = pharmacokinetic; TSH = thyroid-stimulating hormone; WPAI-NASH = Work Productivity and Activity Impairment for Nonalcoholic Steatohepatitis

a Subjects will be screened up to 8 weeks prior to the first dose of study drug. The screening period may be extended by 2 weeks under special circumstances, with prior written approval from the Medical Monitor

b Subjects who do not have a historical ALT and/or AST value available within 28 days to 180 days prior to screening will be required to complete the Pre-baseline visit. The Pre-baseline visit must be performed ≥ 28 days after collecting initial screening laboratory samples

c Subjects may have the option of completing the visit at home using in-home nursing

d Subjects who received at least 1 dose of study drug and prematurely discontinue from the study should complete the ET visit ≤ 5 days of the last dose of study drug

e Subjects will return 30 days after the last dose of study drug to complete the 30-day Follow-up visit

f Physical assessments required to calculate Child-Pugh score are to be completed as part of the physical examination

g Vital sign measurements will include systolic blood pressure, diastolic blood pressure, heart rate, and respiration rate

h Triplicate vital sign measurements will include systolic blood pressure, diastolic blood pressure, and heart rate. Triplicate vital signs will be performed in the same arm with ≥ 2 minutes of rest between measurements. For subjects who agree to participate in the optional PK substudy, additional triplicate vital sign measurements will be required as outlined in Table 10

i Weight should be obtained using a calibrated scale. The subject should be weighed in consistent clothing, as much as possible. On in-clinic dosing days, weight will be collected pre-dose. Weight should be obtained by the same study personnel, as much as possible, across visits for each subject to minimize variability in the measurement

j Abdominal ultrasounds will be performed to monitor liver and gallbladder health

k Subjects must fast ≥ 2 hours prior to each FibroScan® assessment. The equipment for performing FibroScan® examinations should be kept consistent across subject visits

l If historical values for FibroScan® performed ≤ 90 days prior to the Screening visit are available, then the screening FibroScan® examination does not need to be repeated

m The DXA assessment should be performed within ± 7 days of the Week 36 visit

n Assessment should be performed at the ET visit if the subject received at least 16 weeks of treatment. For subjects who discontinued study drug due to a clinical event (except all-cause mortality and liver transplantation) confirmed by the Hepatic Events Adjudication Committee, the assessment should be performed at ET unless medically contraindicated, per Investigator judgment

o A historical biopsy collected within 365 days prior to screening with prior confirmation of fibrosis stage 4 may be accepted as the screening biopsy if the sample is deemed acceptable for interpretation by the central readers
p Liver biopsy to be obtained within −2 weeks/+4 weeks of the Week 36 visit
q For subjects who provide their additional specific consent, MRE assessments will be performed at the timepoints indicated. Subjects must fast ≥ 4 hours prior to each MRE assessment. Refer to Section 6.13 for more information
r The MRE assessment at the Screening visit should be performed 1-14 days prior to the Day 1 visit and after confirming that eligibility criteria have been met. A historical MRE assessment may be accepted for re-screens (refer to Section 6.2 for details)
s The MRE assessment should be performed within ± 5 days of collecting the liver biopsy
t It is recommended that the questionnaire be completed prior to any study procedures being performed and prior to the subject seeing a health care provider. If the baseline questionnaire is missed at the Day 1 visit, the questionnaire should not be collected at the remaining time points
u Subjects will receive counseling regarding lifestyle modifications including the maintenance of a healthy diet and participation in regular exercise at the Screening visit. At each subsequent visit, subjects will be asked if there has been a significant change in diet or physical activity
v Refer to the Laboratory Assessments Table 8 for details
w Samples should be collected in the morning following an overnight fast (no food or drink, except water) of ≥ 8 hours
x Only ELF score will be obtained at screening for subjects who do not have a historical biopsy specimen. If a historical value for an ELF test performed ≤ 90 days prior to the Screening visit is available, then the screening ELF score does not need to be repeated
y Blood samples for PK analysis will be collected pre-dose
z For subjects who provide their additional specific consent, blood samples for the optional PK substudy will be collected as outlined in Table 9 and Table 10
aa Blood samples will be obtained for detection of ADA against EFX. If required, a NAb assay will be run as outlined in Section 6.19.5
bb For subjects who provide their additional specific consent, a blood sample will be collected for the optional genetic substudy
cc For subjects who provide their additional specific consent, blood samples for the optional PBMC substudy will be collected
dd The 24-hour urine collection for the Screening visit should be initiated 1-7 days prior to the Day 1 visit. The window may be extended with prior written approval from the Sponsor
ee Salivary cortisol sample collection at each protocol specified visit should be time matched (± 3 hours) to the Day 1 visit collection
ff For females of childbearing potential only. A serum pregnancy test will be performed at the Screening visit. A urine pregnancy test will be performed at all other visits
gg After obtaining informed consent and prior to initiation of study drug, record any SAEs and AEs related to protocol-mandated procedures
hh Administration of study drug should occur following all other study procedures for that visit

Appendix B. Main Study Schedule of Assessments Table – Long-Term Follow-up

Visit[a]	Week										ET[c]	30-day Follow-up[d]
	40, 44	48	52	56	37, 38, 39, 41, 42, 43, 45, 46, 47, 49, 50, 51, 53, 54, 55, 57, 58, 59, 60, 61, 62, 63[b]	64	72	80, 88	65, 66, 67, 68, 69, 70, 71, 73, 74, 75, 76, 77, 78, 79, 81, 82, 83, 84, 85, 86, 87, 89, 90, 91, 92, 93, 94, 95[b]	96		
Visit Window	±5 days					±7 days					≤ 5 days	±7 days
Complete Physical Examination		X								X	X	
Symptom-driven Physical Examination	X		X	X		X	X	X				X
Child-Pugh Score[e]		X					X			X	X	X
MELD Score	X	X	X	X		X	X	X		X	X	X
Vital Signs[f]	X		X	X		X	X	X				X
Triplicate Vital Signs[g]		X								X	X	
Weight[h]	X	X	X	X		X	X	X		X	X	X
12-lead ECG		X					X			X	X	X
Abdominal Ultrasound[i]		X					X			X	X	
FibroScan®[j]		X					X			X	X	
Dual-energy X-ray Absorptiometry		X[k]								X	X[l]	
Liver Biopsy										X[m]	X[n]	
Optional MRE Substudy[o]										X[p]	X[l,p]	
CLDQ-NASH[q]	X	X	X				X			X	X	
WPAI-NASH[q]	X	X	X				X			X	X	
Lifestyle Guidance[r]	X	X	X	X		X	X	X		X		
Chemistry[s,t]	X	X	X	X		X	X	X		X	X	X
Hematology[s]	X	X	X	X		X	X	X		X	X	X
Coagulation[s]	X	X	X	X		X	X	X		X	X	X
Lipoproteins[s,t]	X	X	X	X		X	X	X		X	X	X

Visit[a]	Week										ET[c]	30-day Follow-up[d]
	40, 44	48	52	56	37, 38, 39, 41, 42, 43, 45, 46, 47, 49, 50, 51, 53, 54, 55, 57, 58, 59, 60, 61, 62, 63[b]	64	72	80, 88	65, 66, 67, 68, 69, 70, 71, 73, 74, 75, 76, 77, 78, 79, 81, 82, 83, 84, 85, 86, 87, 89, 90, 91, 92, 93, 94, 95[b]	96		
Visit Window	±5 days					±7 days					≤ 5 days	±7 days
Serum Storage Samples[s]	X	X	X	X		X	X	X		X	X	X
Plasma Storage Samples[s]	X	X	X	X		X	X	X		X	X	X
Urinalysis[s]		X					X			X	X	
Fibrosis Biomarkers[s]		X					X			X	X	X
Other Biomarkers[s,t]		X					X			X	X	X
PEth Test[s]		X					X			X	X	
EFX PK[s,u]		X		X		X	X	X		X	X	X
ADA and NAb[s,v]		X		X		X	X	X		X	X	X
Endogenous FGF21[s,t]		X		X		X	X	X		X	X	X
Optional PBMC Substudy[s,w]			X							X	X	X
Salivary Cortisol Test[s,x]	X[y]	X		X		X	X	X		X	X	X
IGF-1, Free T4, and TSH Tests[s]		X		X			X	X[z]		X	X	X
Pregnancy Test[s,aa]	X	X	X	X		X	X	X		X	X	X
Adverse Events	X	X	X	X		X	X	X		X	X	X
Concomitant Medications	X	X	X	X		X	X	X		X	X	X
Administer Study Drug[bb]	X	X	X	X	X	X	X	X	X	X		

ADA = anti-drug antibody; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CLDQ-NASH = Chronic Liver Disease Questionnaire-Nonalcoholic Steatohepatitis; ECG = electrocardiogram; EFX = Efruxifermin; FGF21 = fibroblast growth factor 21; IGF-1 = insulin-like growth factor-1; MELD = Model for End-Stage Liver Disease; MRE = magnetic resonance elastography; NAb = neutralizing antibody; PBMC = peripheral blood mononuclear cell; PEth = phosphatidylethanol; PK = pharmacokinetic; TSH = thyroid-stimulating hormone; WPAI-NASH = Work Productivity and Activity Impairment for Nonalcoholic Steatohepatitis

a Study visits will be conducted in-clinic every 4 weeks from Week 40 to Week 56 and every 8 weeks from Week 64 to Week 96 or until end of study

b Subjects may have the option of completing the visit at home using in-home nursing

c Subjects who received at least 1 dose of study drug and prematurely discontinue from the study should complete the ET visit ≤ 5 days of the last dose of study drug

d Subjects will return 30 days after the last dose of study drug to complete the 30-day Follow-up visit

e Physical assessments required to calculate Child-Pugh score are to be completed as part of the physical examination

f Vital sign measurements will include systolic blood pressure, diastolic blood pressure, heart rate, and respiration rate

g Triplicate vital sign measurements will include systolic blood pressure, diastolic blood pressure, and heart rate. Triplicate vital signs will be performed in the same arm with ≥ 2 minutes of rest between measurements

h Weight should be obtained using a calibrated scale. The subject should be weighed in consistent clothing, as much as possible. On in-clinic dosing days, weight will be collected pre-dose. Weight should be obtained by the same study personnel as much as possible across visits for each subject to minimize variability in the measurement

i Abdominal ultrasounds will be performed to monitor liver and gallbladder health

j Subjects must fast ≥ 2 hours prior to each FibroScan® assessment. The equipment for performing FibroScan® examinations should be kept consistent across subject visits

k The DXA assessment should be performed within ± 7 days of the Week 48 visit

l To be performed at ET if the last assessment was completed ≥ 47 weeks prior to the ET visit. For subjects who discontinued study drug due to a clinical event (except all-cause mortality and liver transplantation) confirmed by the Hepatic Events Adjudication Committee, the assessment should be performed at ET unless medically contraindicated, per Investigator judgment

m Liver biopsy to be obtained within −8 weeks/+4 weeks of the Week 96 visit

n Liver Biopsy may be required at ET for adjudication of pre-specified liver-related outcomes or if the previous liver biopsy specimen was obtained ≥ 12 months prior to the ET visit. For subjects who discontinued study drug due to a clinical event (except all-cause mortality and liver transplantation) confirmed by the Hepatic Events Adjudication Committee, the liver biopsy specimen should be obtained at ET unless medically contraindicated, per Investigator judgment

o For subjects who provide their additional specific consent, MRE assessments will be performed at the timepoints indicated. An MRE assessment may not be conducted at the Week 96 visit or Early Termination visit depending on results obtained from the Week 36 primary endpoint analysis. Subjects must fast ≥ 4 hours prior to each MRE assessment. Refer to Section 6.13 for more information

p The MRE assessment should be performed within ± 5 days of collecting the liver biopsy

q It is recommended that the questionnaire be completed prior to any study procedures being performed and prior to the subject seeing a health care provider. If the baseline questionnaire is missed at the Day 1 visit, the questionnaire should not be collected at the remaining time points

r At each visit, subjects will be asked if there has been a significant change in diet or physical activity

s Refer to the Laboratory Assessments Table 8 for details

t Samples should be collected in the morning following an overnight fast (no food or drink, except water) of ≥ 8 hours

u Blood samples for PK analysis will be collected pre-dose

v Blood samples will be obtained for detection of ADA against EFX. If required, a NAb assay will be run as outlined in Section 6.19.5

w For subjects who provide their additional specific consent, blood samples for the optional PBMC substudy will be collected

x Salivary cortisol sample collection at each protocol specified visit should be time matched (± 3 hours) to the Day 1 visit collection

y Salivary cortisol test to be performed at Week 40 only

z IGF-1, Free T4, and TSH tests to be performed at Week 88 only

aa For females of childbearing potential only. A urine pregnancy test will be performed at all visits

bb Administration of study drug should occur following all other study procedures for that visit

Appendix C. Cohort D Schedule of Assessments Table

Visit	Screening[a]	Pre-baseline[b]	Day 1	Week 1	Week 2, 3	Week 4	Week 8	Week 5, 6, 7, 9, 10, 11	Week 12	ET[c]	30-day Follow-up[d]
Visit Window				±3 days						≤ 5 days	±3 days
Written Informed Consent	X										
Eligibility	X	X	X								
Medical History	X										
Complete Physical Examination	X		X						X	X	
Symptom-driven Physical Examination				X		X	X				X
Child-Pugh Score[e]			X						X	X	X
MELD Score			X	X		X	X		X	X	X
Vital Signs[f]	X	X		X	X	X	X				X
Temperature	X		X								
Triplicate Vital Signs[g]			X						X	X	
Height	X										
Weight[h]	X	X	X	X	X	X	X		X	X	X
Hip and Waist Circumference[i]	X		X						X	X	X
12-lead ECG	X		X						X	X	X
FibroScan®[j]	X[k]		X						X	X[l]	
MRI-PDFF[m]	X[n]								X[o]		
Dual-energy X-ray Absorptiometry	X										
Liver Biopsy	X[p]										
Lifestyle Guidance[q]	X		X	X	X	X	X	X	X		
Chemistry[r,s]	X		X	X		X	X		X	X	X
ALT/AST		X									

Visit	Screening[a]	Pre-baseline[b]	Day 1	Week 1	2, 3	4	8	5, 6, 7, 9, 10, 11	12	ET[c]	30-day Follow-up[d]
Visit Window				±3 days						≤ 5 days	±3 days
Hematology[r]	X		X	X		X	X		X	X	X
Coagulation[r]	X		X	X		X	X		X	X	X
Lipoproteins[r,s]	X		X	X		X	X		X	X	X
Serum Storage Samples[r]	X		X	X		X	X		X	X	X
Plasma Storage Samples[r]	X		X	X		X	X		X	X	X
Urinalysis[r]			X						X	X	
Urine Drug Screen[r]	X										
Serology[r]	X										
Fibrosis Biomarkers[r]			X						X	X	X
Other Biomarkers[r,s]			X			X	X		X	X	X
EFX PK[r,t]			X			X	X		X	X	X
ADA and NAb[r,u]			X				X		X	X	X
Endogenous FGF21[r,s]			X								
Optional Genetic Substudy Sample[r,v]			X								
Salivary Cortisol Test[r,w]			X			X	X		X	X	X
TSH, Free T4, and Glucagon Tests[r]			X			X	X		X	X	X
Pregnancy Test[r,x]	X		X			X	X		X	X	X
Adverse Events[y]	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X
Administer Study Drug[z]			X	X	X	X	X	X	X		

ADA = anti-drug antibody; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ECG = electrocardiogram; EFX = Efruxifermin; FGF21 = fibroblast growth factor 21; MELD = Model for End-Stage Liver Disease; MRI-PDFF = magnetic resonance imaging-proton density fat fraction; NAb = neutralizing antibody; PK = pharmacokinetic; TSH = thyroid-stimulating hormone

a Subjects will be screened up to 8 weeks prior to the first dose of study drug. The screening period may be extended by 2 weeks under special circumstances, with prior written approval from the Medical Monitor

b Subjects who do not have a historical ALT and/or AST value available within 28 days to 180 days prior to screening will be required to complete the Pre-baseline visit. The Pre-baseline visit must be performed ≥ 28 days after collecting initial screening laboratory samples

c Subjects who received at least 1 dose of study drug and prematurely discontinue from the study should complete the ET visit ≤ 5 days of the last dose of study drug

d Subjects will return 30 days after the last dose of study drug to complete the 30-day Follow-up visit

e Physical assessments required to calculate Child-Pugh score are to be completed as part of the physical examination

f Vital sign measurements will include systolic blood pressure, diastolic blood pressure, heart rate, and respiration rate

g Triplicate vital sign measurements will include systolic blood pressure, diastolic blood pressure, and heart rate. Triplicate vital signs will be performed in the same arm with ≥ 2 minutes of rest between measurements.

h Weight should be obtained using a calibrated scale. The subject should be weighed in consistent clothing, as much as possible. On in-clinic dosing days, weight will be collected pre-dose. Weight should be obtained by the same study personnel, as much as possible, across visits for each subject to minimize variability in the measurement

i Waist and hip circumference should be obtained by the same study personnel, as much as possible, across visits for each subject to minimize variability in measurement

j Subjects must fast ≥ 2 hours prior to each FibroScan® assessment. The equipment for performing FibroScan® examinations should be kept consistent across subject visits

k If historical values for FibroScan® performed ≤ 90 days prior to the Screening visit are available, then the screening FibroScan® examination does not need to be repeated

l Assessment should be performed at the ET visit if the subject received at least 8 weeks of treatment

m Subjects must fast ≥ 4 hours prior to each MRI assessment. The equipment for performing MRI-PDFF should be kept consistent across subject visits

n The MRI-PDFF assessment at the Screening visit should be performed 1-14 days prior to the Day 1 visit and after confirming that eligibility criteria have been met.

o The MRI-PDFF assessment at the Week 12 visit should be performed within ± 7 days of the Week 12 visit

p A historical biopsy collected within 365 days prior to screening with pathologist confirmation of fibrosis stage 1, 2, or 3 may be accepted as the screening biopsy

q Subjects will receive counseling regarding lifestyle modifications including the maintenance of a healthy diet and participation in regular exercise at the Screening visit. At each subsequent visit, subjects will be asked if there has been a significant change in diet or physical activity

r Refer to the Laboratory Assessments Table 8 for details

s Samples should be collected in the morning following an overnight fast (no food or drink, except water) of ≥ 8 hours

t Blood samples for PK analysis will be collected pre-dose

u Blood samples will be obtained for detection of ADA against EFX. If required, a NAb assay will be run as outlined in Section 6.19.5

v For subjects who provide their additional specific consent, a blood sample will be collected for the optional genetic substudy

w Salivary cortisol sample collection at each protocol specified visit should be time matched (± 3 hours) to the Day 1 visit collection

x For females of childbearing potential only. A serum pregnancy test will be performed at the Screening visit. A urine pregnancy test will be performed at all other visits

y After obtaining informed consent and prior to initiation of study drug, record any SAEs and AEs related to protocol-mandated procedures

z Administration of study drug should occur following all other study procedures for that visit

Appendix D. Pregnancy Precautions, Definition for Female of Childbearing Potential, and Contraceptive Requirements

1) Definitions

a) Definition of Childbearing Potential

For the purposes of this study, a female born subject is considered of childbearing potential following the initiation of puberty (Tanner stage 2) until becoming postmenopausal, unless permanently sterile or with medically documented ovarian failure.

Women are considered to be in a postmenopausal state when they are ≥ 54 years of age with cessation of previously occurring menses for ≥ 12 months without an alternative cause. In addition, women of any age with amenorrhea of ≥ 12 months may also be considered postmenopausal if their follicle-stimulating hormone (FSH) level is in the postmenopausal range and they are not using hormonal contraception or hormonal replacement therapy.

Any testing (e.g., of FSH levels to confirm postmenopausal status) is not mandated by the protocol as a study procedure. It is the responsibility of the Investigator to confirm postmenopausal status.

Permanent sterilization includes hysterectomy, bilateral oophorectomy, or bilateral salpingectomy in a female subject of any age.

b) Definition of Male Fertility

For the purposes of this study, a male born subject is considered fertile after the initiation of puberty unless permanently sterile by bilateral orchidectomy or medical documentation.

2) Contraception Requirements for Female Subjects

a) Study Drug Effects on Pregnancy and Hormonal Contraception

No formal studies have been conducted to evaluate the reproductive toxicity of EFX; therefore, the reproductive toxicity of EFX in humans is unknown.

Efruxifermin has not yet been studied in pregnant women.

Please refer to the latest version of the IB for additional information.

b) Contraception Requirements for Female Subjects of Childbearing Potential

The inclusion of female subjects of childbearing potential requires the use of highly effective contraceptive measures. Female subjects of childbearing potential must have a negative serum pregnancy test at screening and a negative urine pregnancy test prior to randomization on Day 1. Pregnancy tests will be performed as per the Schedule of Assessments (Appendix A,

Appendix B, and Appendix C). Female subjects must agree to one of the following from screening until 30 days after the last dose of study drug.

- Complete abstinence from intercourse of reproductive potential. Abstinence is an acceptable method of contraception only when it is in line with the subject's preferred and usual lifestyle.

  OR

- Consistent and correct use of 1 of the following methods of birth control listed below:

  — Intrauterine device with a failure rate of < 1% per year

  — Intrauterine hormone-releasing system with a failure rate of < 1% per year

  — Tubal sterilization

  — Essure® micro-insert system (provided confirmation of success 3 months after procedure)

  — Vasectomy in the male partner (provided that the partner is the sole sexual partner and had confirmation of surgical success 3 months after the procedure)

Should female subjects wish to use a hormonally based method of contraception, use of a male condom by the female subject's male partner is required. Subjects who utilize a hormonal contraceptive as one of their birth control methods must have used the same method for at least 3 months prior to the first dose of study drug. Hormonally-based contraceptives permitted for use in this protocol are as follows:

— Oral contraceptives (either combined or progesterone only)

— Injectable progesterone

— Implants of levonorgestrel

— Transdermal contraceptive patch

— Contraceptive vaginal ring

Not all of these methods may be approved in each of the countries where the study is being conducted; refer to local product information. Additional local regulatory requirements may apply.

Female subjects must also refrain from egg donation and in vitro fertilization during treatment and until at least 90 days after the last dose of study drug.

3) Contraception Requirements for Male Subjects

It is theoretically possible that a relevant systemic concentration may be achieved in a female partner from exposure of the male subject's seminal fluid. Therefore, male subjects with female partners of childbearing potential must use condoms during treatment until 90 days after the last dose of study drug. Female partners of male study subjects are asked to select 1 of the above methods.

Male subjects must also refrain from sperm donation during treatment and until at least 90 days after the last dose of study drug.

4) Unacceptable Birth Control Methods

Birth control methods that are unacceptable include periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method. Female condom and male condom should not be used together.

5) Procedures to be Followed in the Event of Pregnancy

Subjects will be instructed to notify the Investigator if they become pregnant at any time during the study, or if they become pregnant within 90 days of last study drug dose. Subjects who become pregnant or who suspect that they are pregnant during the study must report the information to the Investigator and discontinue study drug immediately. Subjects whose partner has become pregnant or suspects she is pregnant during the study must report the information to the Investigator. Instructions for reporting pregnancy, partner pregnancy, and pregnancy outcome are outlined in Section 6.23.5.2.

Appendix E. West Haven Criteria

http://www.mdcalc.com/hepatic-encephalopathy-grades-stages/

Grade of Hepatic Encephalopathy	Description	Suggested Operative Criteria
Grade I	• Trivial lack of awareness • Euphoria or anxiety • Shortened attention span • Impairment of addition or subtraction • Altered sleep rhythm	Despite oriented in time and space (see below), the subject appears to have some cognitive/behavioral decay with respect to his or her standard on clinical examination or to the caregivers
Grade II	• Lethargy or apathy • Disorientation for time • Obvious personality change • Inappropriate behavior • Dyspraxia • Asterixis	Disoriented for time (at least 3 of the following are wrong: day of the month, day of the week, month, season, or year) ± the other mentioned symptoms
Grade III	• Somnolence to semi stupor • Responsive to stimuli • Confused • Gross disorientation • Bizarre behavior	Disoriented also for space (at least 3 of the following wrongly reported: country, state [or region], city, or place) ± the other mentioned symptoms
Grade IV	• Coma	Does not respond even to painful stimuli

Source: Vistrup H, AModio P, Bajaj J, Cordoba J, Ferenci P, Mullen KD et al. Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver. Hepatology. 2014;60(2):715–35

Appendix F. Child-Pugh Score

Child-Pugh class is obtained by adding the score for each parameter (total points) in Table 13.

- Class A = 5 to 6 points (least severe liver disease)
- Class B = 7 to 9 points (moderately severe liver disease)
- Class C = 10 to 15 points (most severe liver disease)

Table 13. Child-Pugh Score Components

Clinical[a] and Lab Criteria	1 point	2 points	3 points
Encephalopathy[b]	None	Mild to Moderate (Grade 1 or 2)	Severe (Grade 3 or 4)
Ascites[b]	None	Mild to Moderate (diuretic responsive)	Severe (diuretic refractory)
Bilirubin (mg/dL)	< 2	2—3	> 3
Albumin (g/dL)	> 3.5	2.8—3.5	< 2.8
International normalized ratio	< 1.7	1.7—2.3	> 2.3

a Physical assessments required to calculate Child-Pugh score are to be completed as part of the physical examinations

b Records of concomitant medications for encephalopathy and ascites will be recorded in the eCRF

Appendix G. Definitions of Compensated Cirrhosis Due to Nonalcoholic Steatohepatitis (NASH)

Table 14. Definition of Terms

Definitive NASH	Cryptogenic Cirrhosis Presumed Secondary to NASH
Histologic evidence of steatohepatitis with a NAS ≥ 3 and ≥ 1 point each in steatosis, lobular inflammation, and ballooning	No clear evidence of steatosis or steatohepatitis on histology (potentially due to burn-out), but no evidence of competing etiology and one of the following: previous history or presence of T2D or 2 out of 4 components of metabolic syndrome (obesity, dyslipidemia, elevated blood pressure, elevated fasting glucose)

NAS = NAFLD Activity Score; NASH = nonalcoholic steatohepatitis; T2D = type 2 diabetes

Modified From: Noureddin M, Chan JL, Barradas K, Dimick-Santos L, Schabel E, Omokaro SO, Anania FA, Myers RP, Miller V, Sanyal AJ, Chalasani N; Liver Forum NASH Cirrhosis Working Group. Attribution of Nonalcoholic Steatohepatitis as an Etiology of Cirrhosis for Clinical Trials Eligibility: Recommendations From the Multi-stakeholder Liver Forum. Gastroenterology. 2020 Aug;159(2):422-427