CLINICAL STUDY PROTOCOL:
A PHASE 2B, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY EVALUATING THE SAFETY AND EFFICACY OF EFRUXIFERMIN IN NON-CIRRHOTIC SUBJECTS WITH NONALCOHOLIC STEATOHEPATITIS (NASH)

Protocol Number: AK-US-001-0102

IND Number: 140307

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: Amendment 4, 18 Apr 2022

STUDY ACKNOWLEDGEMENT SIGNATURE PAGE

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

AK-US-001-0102, Amendment 4, 18 Apr 2022

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

[redacted]

4/22/2022
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 drugs 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) 28 mg and 50 mg

Title of Study:

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

Investigator and Study Centers:

Approximately 55 centers in North America

Study Period:

Participation will include:

- Screening: 8 weeks
- Primary Endpoint Treatment Duration: 24 weeks
- Long-Term Follow-Up: Up to 96 weeks

Phase of Development:

Phase 2b

Objectives:

The primary objective of this study is:

- To evaluate the effect of EFX compared to placebo on fibrosis regression in NASH subjects with stage 2 or 3 fibrosis.

The secondary objectives of this study are:

- To evaluate the effect of EFX compared to placebo on achieving NASH resolution in subjects with stage 2 or 3 fibrosis.
- 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 glycemic control.
- To evaluate the effect of EFX compared to placebo on weight change.
- To assess the safety, tolerability, and immunogenicity of EFX.

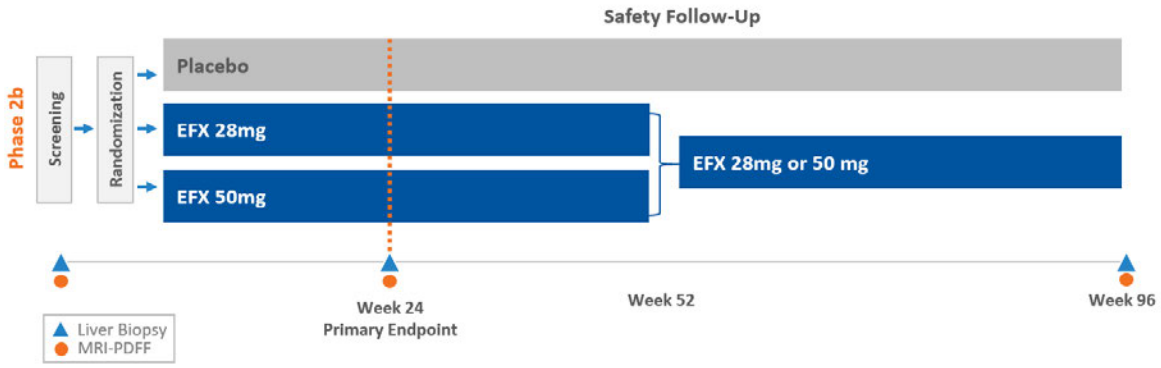
Exploratory objectives are outlined in the protocol in Section 2.3.

Study Design:

This is a Phase 2b, randomized, double-blind, placebo-controlled study evaluating the safety and efficacy of EFX in non-cirrhotic subjects with NASH. Subjects will be fibrosis stage 2 or 3 (F2-3) and dosed weekly with EFX or placebo.

Subjects meeting the study's eligibility criteria will be randomly assigned in a 1:1:1 ratio into 3 treatment groups as shown in the figure below. Following 24 weeks of dosing, subjects will transition into the long-term safety follow-up portion of the study and will be followed until Week 96. If a subject refuses to complete the Week 24 liver biopsy, the subject will be discontinued from the study at Week 24. Only a 30-Day Follow-up visit is required following discontinuation of study drug. After all subjects have completed at least 52 weeks of treatment on their randomized dose, all subjects randomized to receive EFX will roll over into a single EFX dose arm (28 mg or 50 mg). The dose will be selected based on the Week 24 analysis and in alignment with regulatory authority.

Subjects on placebo will remain in the same treatment group for the duration of the study. After completing Week 96, subjects on placebo will be offered the option to receive EFX in a rollover study.



Randomization:

Randomization will be stratified by baseline type-2 diabetes status and fibrosis score.

Study drug will be administered subcutaneously once weekly (QW).

Eligible subjects will be randomized to one of three treatment groups with a ratio of 1:1:1

Group A: Placebo

Group B: 28 mg EFX

Group C: 50 mg EFX

Number of Subjects Planned:

Approximately [redacted] subjects

Target Population:

Males and non-pregnant, non-lactating females between 18 – 75 years of age with biopsy proven F2 - F3 NASH.

Diagnosis and Main Criteria for Inclusion:

Inclusion Criteria

1. Males and non-pregnant, non-lactating females between 18 - 75 years of age inclusive, at the time of the screening visit.
2. Previous history or presence of 2 out of 4 components of metabolic syndrome (obesity, dyslipidemia, elevated blood pressure, elevated fasting glucose) or type 2 diabetes.
3. **Initial Screening Visit**: After a signed informed consent and confirmation of clinical risk profile associated with NASH, a screening lab panel and elastography will be measured for all subjects during an initial screening period:
    a. FibroScan® median liver stiffness [redacted]
    b. CAP [redacted]
    Note: All subjects must complete a FibroScan® examination during the screening period. However, the median liver stiffness and CAP inclusion criterion do not apply to subjects

with an eligible historical liver biopsy performed ≤ 180 days prior to randomization which confirmed fibrosis 2-3 and a NAS ≥ 4.

If a historical value for FibroScan® is available in the past 3 months prior to the Screening Visit, then the FibroScan® does not need to be repeated.

c. AST > 17 for females and > 20 for males;
Note: The AST inclusion criterion does not apply to subjects with an eligible historical liver biopsy performed ≤ 180 days prior to randomization which confirmed fibrosis 2-3 and a NAS ≥ 4.
d. Estimated glomerular filtration rate (eGFR) ≥ 60 mL/min, as calculated by the CKD-EPI;
e. HbA1c ≤ 9.5% (or serum fructosamine ≤ 381 µmol if HbA1c is unavailable);
f. INR ≤ 1.3, unless due to therapeutic anticoagulation;
g. Direct bilirubin ≤ ULN;
h. Total bilirubin ≤ upper limit of normal (ULN), unless due to an alternate etiology such as Gilbert's syndrome or hemolytic anemia;
i. Creatinine kinase < 3 x ULN;
j. Platelet count ≥ 140,000/µL;
k. Triglyceride level ≤ 500 mg/dL;
l. ALT < 5 x ULN;
m. AST < 5 x ULN;
n. ALP < 2 x ULN.

Note: Subjects meeting all of the above components will move into the next screening phase.

4. Documented historical stability (4 weeks to 6 months prior to screening) of ALT and AST levels showing no worsening at screening based on the following:
   a. If the historical and screening ALT and AST values are both ≤ 1.5 × the upper limit of normal (ULN), there is no limit to the difference between the values.
   b. If at least 1 of the historical values of ALT or AST is >1.5 × ULN and shows worsening at screening, the difference of ALT and AST values must be ≤ 50%.

   Note: Subjects without historical ALT and AST evaluations may have ALT and AST repeated (Pre-Baseline Visit) during the screening period at minimum 4 weeks apart to confirm a or b above;

Subjects who are ineligible at the screening or pre-baseline visit may be re-screened or have an assessment repeated once if there is reasonable belief that the exclusionary result was obtained in error or is transient upon approval of the Medical Monitor.

5. MRI-PDFF [redacted]

   Note: All subjects must complete an MRI-PDFF examination during the screening period. However, the MRI-PDFF inclusion criterion does not apply to subjects with an eligible historical liver biopsy performed ≤ 180 days prior to randomization which confirmed fibrosis 2-3 and a NAS ≥ 4.

6. Biopsy-proven NASH. Must have had a liver biopsy obtained ≤ 180 days prior to randomization with fibrosis stage 2 to 3 and a non-alcoholic fatty liver disease (NAFLD) activity score (NAS) of ≥ 4 with at least a score of 1 in each of the following NAS components:
   a. Steatosis (scored 0 to 3),
   b. Ballooning degeneration (scored 0 to 2), and
   c. Lobular inflammation (scored 0 to 3)

7. Subjects on Vitamin E ≥ 400 IU/day, thiazolidinediones (including, but not limited to, pioglitazone, rosiglitazone, and lobeglitazone), GLP-1 agonists, or SGLT2 inhibitors must be on a stable dose (defined as no significant change in prescription efficacy, initiation of medication, or medication discontinuation) for at least 3 months prior to the diagnostic liver biopsy through randomization. A switch from one drug to another in the same class should be discussed with the Medical Monitor to confirm eligibility.
8. Subjects on antidiabetic, weight loss, or lipid-modifying medication(s) must be on stable dose(s) for at least 3 months prior to the diagnostic liver biopsy through randomization;
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 pregnancy test at screening and 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:

1. Weight loss > 5% in the 3 months prior to screening until randomization or from the time of the diagnostic liver biopsy until randomization, whichever is longer.
2. Presence of cirrhosis on liver biopsy (stage 4 fibrosis).
3. Type 1 diabetes.
4. Uncontrolled Type 2 diabetes defined as:
   a. Insulin dose adjustment >35% within 30 days prior to screening through randomization,
   b. Symptoms of the following within 3 months prior to screening: acutely decompensated blood glucose control (e.g., thirst, polyuria, weight loss), or a history of diabetic ketoacidosis, or a history of hyperglycemic hyperosmolar state;
5. Hypoglycemia unawareness, hospitalization due to hypoglycemia, or history of severe hypoglycemia (hypoglycemia requiring outside assistance to regain normal neurologic status) within 3 months prior to screening.
6. Subjects with osteoporosis, defined as a T-score of -2.5 or lower at screening.
7. Poorly controlled hypertension (systolic > 160 mm Hg, or diastolic blood pressure > 100 mm Hg).
8. Any prior history of decompensated liver disease including ascites, hepatic encephalopathy (HE), or variceal bleeding.
9. History of pancreatitis.
   Note: Subjects with a history of gallstone pancreatitis and subsequent cholecystectomy may be allowed to participate upon approval by the Medical Monitor.
10. Chronic hepatitis B virus (HBV) infection (hepatitis B surface antigen [HBsAg]) positive or acute hepatitis A infection (hepatitis A IgM antibody positive).
11. Chronic hepatitis C virus (HCV) infection (HCV antibody [Ab] and HCV ribonucleic acid [RNA] positive). Subjects cured of HCV infection < 2 years prior (based on date of RNA polymerase chain reaction [PCR] negative confirmation following conclusion of treatment) to the screening visit are not eligible.
12. Prior (< 2 years prior to screening) or planned (during the study period) bariatric surgery (e.g., gastroplasty, roux-en-Y gastric bypass). Surgery failure or reversal or removal of intragastric balloon > 2 years prior to screening would be acceptable.
13. Other causes of liver disease based on medical history and/or centralized review of liver histology, 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 requiring treatment.
14. History of liver transplantation.
15. Current or prior history of hepatocellular carcinoma (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 average of >20 g/day in female subjects and >30 g/day in male subjects.

17. Human immunodeficiency virus (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 the screening electrocardiogram (ECG) assessment;
19. Myocardial infarction, unstable angina, percutaneous coronary intervention, coronary artery bypass graft, or stroke within 3 months prior to screening.
20. Life expectancy less than 2 years.
21. Use of any investigational medication within 30 days or within 5 half-lives prior to screening or concurrent participation in another therapeutic clinical study. Participation in an experimental vaccine trial (e.g., COVID-19 or other) may be acceptable upon approval by the medical monitor.
22. Subjects with a history of (within 12 months prior to screening) or current use of prescription drugs associated with liver steatosis (e.g., methotrexate, amiodarone, high-dose estrogen, tamoxifen, systemic steroids, anabolic steroids, valproic acid). Short courses of systemic corticosteroids (less than two weeks) and 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;
23. Positive urine drug screen for amphetamines, cocaine or opiates (e.g., heroin, morphine) at screening. Subjects on stable methadone or buprenorphine maintenance treatment for at least 6 months prior to screening may be included in the study. 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.
24. Unable to safely undergo a liver biopsy.
25. Subjects who have contraindications to MR imaging (e.g., unmanageable claustrophobia, certain metal implants, or unable to fit within MR scanner due to girth).
26. Presence of any laboratory abnormality or significant systemic or major illnesses (other than liver disease) that, in the opinion of the Investigator, compromise 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 six months of screening.
27. Unavailable for follow-up assessment or concern for subject's compliance with the protocol procedures.

Investigational Product, Dosage, and Mode of Administration:

EFX is an IgG1Fc-fibroblast growth factor 21 (FGF21) fusion protein.

EFX or Placebo will be provided as a frozen liquid formulation and is administered subcutaneously in the abdomen as follows:

- Placebo QW (n = 40)
- 28 mg EFX QW (n = 40)
- 50 mg EFX QW (n = 40)

Criteria for Evaluation:

Safety:

The safety of EFX will be assessed during the study through the reporting of adverse events (AEs), clinical laboratory tests, electrocardiogram (ECG), Bone Mineral Density (BMD), 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 two hepatologists, one cardiologist, and a statistician will review the progress of the study. The DMC will convene after 45 subjects (approximately 15 per treatment group) have completed the Week 12 assessments. The DMC will receive all reports of serious

adverse events (SAEs), potential drug-induced liver injury (DILI) events for evaluation and convene as needed to monitor for safety and additional meetings will be scheduled as required.

Throughout the study, all treatment-emergent adverse events (TEAEs), clinical assessments, clinical laboratory parameters, and potential DILI will be closely monitored with subjects entering close observation, as required.

Pharmacokinetics:

Blood for pharmacokinetics (PK) will be collected at specified timepoints after dosing as outlined in the Schedule of Assessments (Schedule of Assessments) 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 subcutaneous (SC) administration of EFX.

Efficacy:

The primary efficacy endpoint at Week 24 includes the proportion of subjects who achieve improvement in liver fibrosis, defined as ≥ one stage (NASH CRN fibrosis score) and no worsening of steatohepatitis (defined as no increase in NAS for ballooning, inflammation, or steatosis).

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 resolution of steatohepatitis (defined as a NAS of 0-1 for inflammation, 0 for ballooning, and any value for steatosis) and no worsening of liver fibrosis as determined by the NASH CRN criteria at Week 24 and Week 96.
- Proportion of subjects who achieve improvement in liver fibrosis, defined as ≥ one stage (NASH CRN fibrosis score) and no worsening of steatohepatitis (defined as no increase in NAS for ballooning, inflammation, or steatosis) at Week 96.
- Proportion of subjects who achieve improvement in liver fibrosis, defined as ≥ one stage (NASH CRN fibrosis score) at Week 24 and Week 96.
- Change from baseline in hepatic fat fraction measured by MRI-PDFF at Week 24 and Week 96.
- Change from baseline of lipoproteins – triglycerides, Non-HDL-C, HDL-C and LDL-C.
- Change from baseline of markers of glycemic control – HbA1c, C-peptide, Adiponectin and HOMA-IR.
- Change from baseline in non-invasive markers of fibrosis – ELF, Pro-C3, C3M, NIS-4 and liver stiffness assessed by transient elastography (FibroScan®).
- Change from baseline of body weight.

Liver biopsies will be sent to a central laboratory and then read by two independent pathologists in a blinded manner. Week 24 and end of treatment biopsies will be combined with baseline biopsies and read by both pathologists in a blinded 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.

The DMC will monitor study progress, tracking the actual annual event rate for the outcomes endpoint compared with the assumed prevalence rate per year.

Sample Size Calculation:

The primary endpoint is the percentage of subjects with improvement in liver fibrosis ≥ one stage and no worsening of steatohepatitis at Week 24. It is predicted [redacted] of subjects will reach this endpoint for the placebo group, based on published results. Based on phase 2a BALANCED study of EFX it is estimated that approximately [redacted] subjects of each EFX dose group will reach this endpoint. With two-sided Pearson chi-square test for proportion difference, at a significance level of [redacted] completed subjects per group will provide at least [redacted] power. Factoring in dropouts, the study plans to randomize at least [redacted] subjects in total ([redacted] subjects per group).

Statistical Methods:

Population:	The analysis set for the non-histological efficacy analyses will be the Full Analysis Set (FAS), which includes all subjects who were randomized to the study. 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 Set which includes all subjects who received at least one dose of study drug.
Efficacy Analysis:	**Primary Efficacy Analysis:** A Cochran-Mantel-Haenszel (CMH) test will be used to compare the differences in proportion of subjects who achieve a ≥ 1-stage improvement in fibrosis without worsening of steatohepatitis at Week 24 (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 and no worsening of liver fibrosis, the same 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 will be used instead.
Pharmacokinetic Analysis:	Plasma concentrations of EFX will be listed and summarized.

Detailed description of data analysis and statistical methods to be used will be outlined separately in a Statistical Analysis Plan (SAP).

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

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ADA	anti-drug antibodies
AE	adverse event
AESI	adverse event of special interest
ALP	alkaline phosphatase
ALT	alanine aminotransferase (SGPT)

ANC	absolute neutrophil count
ANCOVA	analysis of covariance
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase (SGOT)
AUC	area under the time-concentration curve
BMI	body mass index
BSAP	bone specific alkaline phosphatase
BUN	blood urea nitrogen
CAP	controlled attenuation parameter
CFR	Code of Federal Regulations
CI	confidence interval
C_{max}	maximum plasma drug concentration
C_{min}	minimum plasma drug concentration
CP	Child-Pugh
CRF	case report form
CRN	Clinical Research Network
CRO	Clinical Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
CV	coefficient of variation
DILI	drug-induced liver injury
DNA	deoxyribonucleic acid
DXA	Dual Energy X-ray Absorptiometry
ECG	electrocardiogram
eCRF	electronic Case Report Form
EDC	Electronic Data Capture
EFX	efruxifermin
eGFR	estimated Glomerular Filtration Rate
EIU	Exposure in Utero
ELF™	Enhanced Liver Fibrosis Panel

EU	European Union
FAS	full analysis set
FDA	Food and Drug Administration
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
FVB	Friend Virus B
GCP	Good Clinical Practice
GGT	gamma glutamyl transferase
GLP	Good Laboratory Practice
HbA1c	hemoglobin A1c
HBcAb	total hepatitis B core antibody
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
Hct	hematocrit
HCV	hepatitis C virus
HDL	high-density lipoprotein cholesterol
Hgb	hemoglobin
HIPAA	Health Information Portability and Accountability Act
HITECH	Health Information Technology for Economic and Clinical Health
HIV	human immunodeficiency virus
HLGT	high-level group term
HLT	high-level term
HOMA	homeostasis model assessment of insulin resistance, (HOMA-IR) and beta cell function (HOMA-B)
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IgG1	human immunoglobulin G1

IMP	investigational medicinal product
IND	Investigational New Drug
INR	International Normalized Ratio
IP	investigational product
IRB	Institutional Review Board
IRT	interactive response technology
ITT	intent-to-treat
IV	intravenous
KO	knock-out
LBAS	liver biopsy analysis set
LDH	lactate dehydrogenase
LDL	low-density lipoprotein cholesterol
LLT	low-level term
LOCF	last observation carried forward
mAb	monoclonal antibody
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
MRE	magnetic resonance elastography
MRI	magnetic resonance imaging
MRI-PDFF	magnetic resonance imaging – proton density fat fraction
NAFLD	non-alcoholic fatty liver disease
NA	North America
NAS	non-alcoholic fatty liver disease activity score
NASH	nonalcoholic steatohepatitis
NOAEL	no-observed-adverse-effect-level
PBC	primary biliary cholangitis
PD	pharmacodynamics
PI	principal investigator
PK	pharmacokinetics

PSC	primary sclerosing cholangitis
PT	preferred term
PT	prothrombin time
Q2W	every 2 weeks
QW	every week
RBC	red blood cell (count)
RNA	ribonucleic acid
SC	subcutaneous
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	standard deviation
SE	standard error
SGOT	serum glutamate oxaloacetate transaminase (AST)
SGPT	serum glutamate pyruvate transaminase (ALT)
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
$T_{1/2}$	time to half plasma concentration (half-life)
TBL	Total bilirubin
TEAEs	treatment emergent adverse events
TESAEs	treatment-emergent serious AEs
TK	toxicokinetics
T_{max}	time to maximum plasma concentration
TSH	thyroid stimulating hormone
ULN	upper-limit of normal
U.S.	United States
WBC	white blood cell (count)

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 and major adverse cardiac events (Ekstedt et al. 2015), underlining the healthcare burden presented by NASH in the absence of approved therapies.

NASH 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). NASH can potentially progress to advanced liver disease, cirrhosis, and hepatocellular carcinoma (The Nash Education Program 2019). The current prevalence of NASH in the United States (U.S.) population is estimated to be around 3 – 4%. Approximately 1 in 5 of the nearly 80 million patients with NAFLD in the U.S. (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. FGF21

Fibroblast growth factor 21 (FGF21) is a member of the endocrine FGF sub-family. FGF21 forms a ligand/co-receptor complex by first anchoring to β-Klotho on the cell surface, then interacting with one of three members of the 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). FGF21 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). FGF21 directly modulates whole-body lipid metabolism to reduce hepatic lipid accumulation. FGF21 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 lipid into the liver, and the ensuing downstream sequelae of hepatocyte stress leading to hepatic inflammation and fibrosis. FGF21 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).

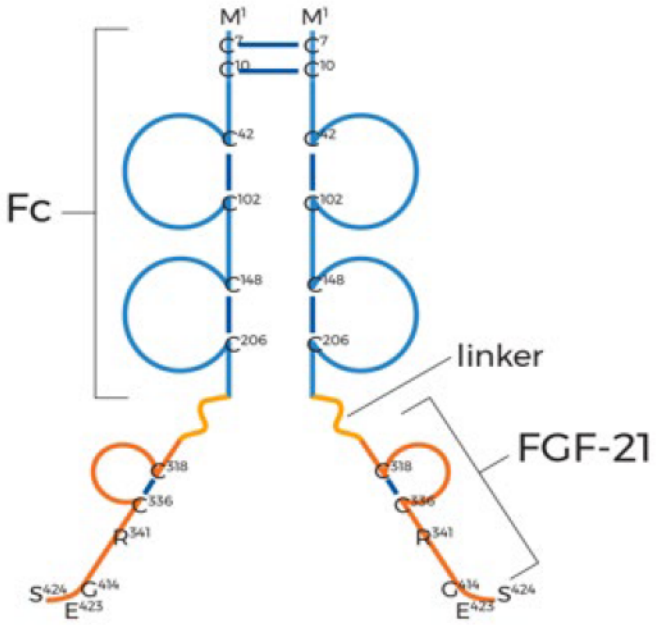
Various in vitro and in vivo findings suggest potential beneficial effects of FGF21 agonism in NASH, including improvements in liver fat, inflammation, and fibrosis (Xu et al. 2009, Lee et al. 2016, Tanaka et al. 2015, Cui et al. 2020). Administration of recombinant human FGF21 or long-acting FGF21 analogues 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. FGF21 knockout (KO) mice are viable, fertile, and show no strong metabolic phenotypes when fed a normal chow diet. However, they become insulin resistant with hepatosteatosis and have significantly elevated plasma glucose and triglyceride 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

EFX is a 92.0 kD *E. coli*-expressed human IgG_1 Fc-FGF21 fusion polypeptide—a long-acting FGF21 analog. Each molecule contains one dimeric Fc domain and 2 modified FGF21 chains. EFX has 8 disulfide bonds, 6 intra-chain, and 2 inter-chain as depicted in Figure 1. Two of the intrachain disulfide bonds are in the FGF21 polypeptide between Cys318 and Cys336, one for each monomer. Additionally, three 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.

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



1.3. Nonclinical Pharmacology & Toxicology

In vitro characterization of EFX demonstrated high affinity binding (<10nM) to human, cynomolgus monkey, and mouse β-Klotho. EFX 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 triglyceride levels; improved lipoprotein profile; enhanced glucose tolerance; and significant weight loss (Stanislaus et al. 2017).

EFX has been evaluated in 16- and 26-week nonclinical toxicology studies in rats and monkeys administered subcutaneous doses of 0 (vehicle control), 10, 30, or 100 mg/kg once weekly. 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 ALT and or 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. EFX-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; 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 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 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 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 50 mg, and corresponding $AUC_{0\text{-}96h}$ values was 11-fold higher than the modeled $AUC_{0\text{-}168h}$ in humans at 50 mg. The exposure multiples based on 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 or carcinogenicity studies have not been conducted to date with EFX. A 2-year rat carcinogenicity study will be conducted in parallel with this program. 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 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 once weekly at up to 100 mg/kg. In addition, there were no pre-neoplastic histopathology changes observed in rats or in monkeys administered weekly subcutaneous doses up to 100 mg/kg/dose for 26 weeks. These data suggest that EFX would 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/dose, nor did it impair fertility of males at doses up to 100 mg/kg/dose. 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. EFX was not teratogenic in rats or rabbits.

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

1.4. Clinical Trials of Efruxifermin

As of 01 October 2020, EFX has been evaluated in Phase 1 single- and multiple-ascending dose studies up to 4-weeks treatment duration in type 2 diabetes (T2D) subjects as well as one Phase 2a study in NASH subjects. In the Phase 1a single-ascending dose study, the highest dose tested was 210 mg administered subcutaneously. In the Phase 1b multiple-ascending dose study, the highest dose tested was 140 mg QW SC. In the Phase 2a study, 28, 50, and 70 mg doses were evaluated at 16-weeks of treatment. In all studies, the drug was generally well tolerated. 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 to evaluate the safety and efficacy of EFX in subjects with NASH. The study consisted of the Main Study and a Cohort C. All results included in Section 1.4.1 includes data from the Main Study only.

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. Subject Disposition

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

A total of 80 subjects were enrolled with 79 subjects receiving at least one dose of study drug. A total of 66 subjects (82.5%) completed investigational product as per the dosing regimen for 16 weeks. Thirteen subjects (16.3%) discontinued investigational product (4 subjects in 70 mg EFX, 4 subjects in 50 mg EFX, 3 subjects in 28 mg EFX and 2 subjects in the placebo group). Seven subjects discontinued investigational product because of AEs and 7 subjects (9.0%) discontinued the study due to administrative reasons. A total of 66 subjects (82.5%) completed the study.

1.4.1.2. Safety Results

Overall, 70 (88.6%) subjects experienced any 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 TESAE of pancreatitis acute and subsequently, diabetic ketoacidosis. This subject was considered morbidly obese and had severe insulin resistance as indicated by acanthosis nigricans and hyperinsulinemia prior to starting the study.

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 in the 70 mg EFX group. There was one study drug-related TESAE and no deaths due to TEAEs.

Overall, 2 (2.5%) subjects experienced an 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 pancreatitis acute and subsequently, diabetic ketoacidosis. This subject was considered morbidly obese and had severe insulin resistance as indicated by acanthosis nigricans and hyperinsulinemia prior to starting the study.

In the subjects receiving at least one dose of EFX, 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 fibroblast growth factor 21 (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.

1.4.1.3. Efficacy Results

All three EFX dose groups 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 treatment groups respectively ($p<0.0001$ for each). Among subjects receiving placebo, a 0.3 reduction ($p = 0.3828$) was observed.

All three 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 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).

At Week 12, 11 (57.9%), 17 (85.0%), and 14 (70.0%) subjects in the 28, 50, and 70 mg EFX groups, respectively, and 1 (4.8%) subject in the placebo group (who had a weight loss of 25 pounds over 16 weeks; 11% weight reduction) had a ≥50% reduction in hepatic fat fraction. There was a significant difference ($p\leq0.0030$) between each EFX treatment group and placebo in the number of subjects with a ≥50% reduction in hepatic fat fraction for the FAS.

When analyzing the MRI-PDFF Evaluable Analysis Set (defined as subjects who had baseline and Week 12 hepatic fat fraction assessed by MRI-PDFF) at Week 12, 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. 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 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***	-13.4***	-14.1***
Normalization (<5%) of hepatic fat fraction[b] (%)	5	21	45	50**
Relative reduction in hepatic fat fraction[a] (%)	0	-63***	-71***	-72***
≥30% Relative reduction in hepatic fat fraction[b] (%)	10	84***	85***	75***
≥50% Relative reduction in hepatic fat fraction[b] (%)	5	58**	85***	70***
Reduction in ALT[a] (U/L)	-6	-24***	-30***	-32***

[a] Least-squares mean change from baseline
[b] Least-squares mean proportion of subjects
** $p<0.004$, versus placebo
*** $p<0.001$, versus placebo

Subjects who achieved a ≥30% reduction of hepatic fat fraction after 12 weeks treatment, were eligible for a biopsy after 16 weeks 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, 28 mg, 50 mg, and 70 mg 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 NAS was defined as a subject with a decrease of ≥2 points in NAS with at least 1-point reduction in either lobular inflammation or hepatocellular ballooning with no concurrent worsening of fibrosis stage. In each of the 28 mg and 50 mg groups, 76.9% of subjects were responders. In the 70 mg group, 78.6% of subjects were responders (Table 2). Of the 40 EFX subjects in the Liver Biopsy Evaluable Analysis Set, 53.8%, 76.9% and 35.7% in the 28 mg, 50 mg, and 70 mg EFX groups, respectively, had ≥1 stage improvement in fibrosis regression (Table 2). Neither of the placebo responders had regression of fibrosis.

Of the same 40 EFX subjects, a total of 11 (27.5%) achieved ≥2 stage improvement in fibrosis regression. This included 4 subjects (30.8%) in the 28 mg group, 5 subjects (38.5%) in the 50 mg group, and 2 subjects (14.3%) in the 70 mg group.

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 46.2%, 53.8%, and 42.9% respectively of the 28-, 50-, and 70-mg groups (Table 2). One of two placebo subjects met this endpoint. Overall, of the 40 EFX subjects, 19 (47.5%) experienced resolution of NASH without worsening of fibrosis stage.

The proportion of subjects who achieved NASH resolution and improvement in fibrosis of ≥1 stage was 30.8%, 38.5%, and 14.3% of subjects respectively in the 28-, 50-, and 70-mg EFX groups, respectively. Across the 40 EFX subjects, 11 (27.5%) achieved NASH resolution and improvement of ≥1 stage of fibrosis (Table 2). Neither placebo subject met this endpoint.

Overall, 19 (47.5%) subjects achieved improvement in liver fibrosis ≥1 stage and no worsening of steatohepatitis. Six (46.2%), 8 (61.5%), and 5 (35.7%) subjects in the 28, 50, and 70 mg EFX groups, respectively, and 0 (0.0%) subjects in the placebo group achieved improvement in liver fibrosis ≥1 stage and no worsening of steatohepatitis. Of all EFX-treated subjects, 55.0% experienced fibrosis improvement of ≥1 stage.

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

Measure (Mean)	Placebo (N=2)	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 (%)[1]	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 (%)[1]	0 (0)	4 (30.8)	5 (38.5)	2 (14.3)
NASH resolution and no worsening of fibrosis stage (%)[1]	1 (50.0)*	6 (46.2)	7 (53.8)	6 (42.3)
NASH resolution AND improvement in fibrosis stage (%)[1]	0 (0)	4 (30.8)	5 (38.5)	2 (14.3)

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

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

The overall metabolic effect of EFX was impressive, and healthy lipoprotein profiles were restored in subjects presenting with dyslipidemia. Significant changes at Week 16 were

observed across all EFX groups in 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 triglycerides (decreases ranging from 71.03 to 89.62 mg/dL). Most importantly, there were no increases in 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, including the first report of significant weight loss in 1 cohort. Significant reductions at Week 16 were observed in HbA1c for the 50 and 70 mg dose groups (-0.39% and -0.50%, respectively). Clinically meaningful improvements were also observed for insulin resistance as measured by 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 ELF Score, which is comprised of HA, TIMP-1 and amino terminal Pro-C3; and Pro-C3, a marker of new collagen synthesis or fibrogenesis.

There was a significant reduction from baseline to Week 12/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 ($p\leq0.0038$).

1.4.1.4. 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 F-stage and no worsening of NASH, or resolving NASH and no worsening of fibrosis after 16 weeks of treatment.

EFX also ameliorated two 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.

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

1.5. Pharmacokinetic Results

In type-2-diabetics, following a single dose, the median time to reach C_{max} ranged from 2 to 5 days. Following SC dosing, mean EFX area under the drug concentration versus time curve (AUC) values increased 309-fold over the 100-fold dose range. The mean EFX $t_{1/2}$ for the SC cohorts was approximately 3.3 days. The mean $t_{1/2,z}$ was approximately 2.62 days after intravenous (IV) dosing. Overall, the PK of EFX demonstrated slightly supra-proportional exposure.

In type-2-diabetics, following multiple doses, EFX exhibited approximately dose proportional PK after Q2W or QW SC administration of 7 mg to 140 mg. The median T_{max} values ranged from 2 to 3.5 days for all cohorts. EFX C_{max} and AUC increased dose-proportionally from 7 mg to 140 mg after Q2W or QW SC administration. Minimal accumulation was observed following Q2W dosing. Following QW dosing, moderate accumulation was observed, with mean accumulation ratios ranging from 1.56 to 3.61. No trend between dose and accumulation ratio was observed.

In NASH subjects, EFX exhibited approximately dose-proportional PK after QW administration of 28 to 70 mg; noncompartmental parameters for NASH subjects were estimated by a population PK model. Relative to type-2-diabetics, NASH subjects exhibited approximately 4-fold lower clearance yielding a half-life of approximately 9 days and an accumulation ratio of approximately 4-fold. Due to the apparent decrease in clearance, C_{max} and AUC are similarly increased approximately 4-fold in NASH subjects relative to the type-2-diabetics.

1.6. Rationale for this Study

NAFLD 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 forecast that by 2030 there will be more than 8 million patients with late-stage NASH in the U.S. (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 (Angulo et al. 2015).

EFX 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). EFX has been engineered to increase human FGF21's half-life (t1/2) (up to 3.5 days) sufficiently to enable once weekly (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 demonstrates 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 resolution of steatohepatitis and crucially 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. 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 of treatment.

This is a Phase 2b, randomized, double-blind, placebo-controlled study evaluating the safety and efficacy of EFX in non-cirrhotic subjects with NASH. Subjects will be fibrosis stage 2 or 3 (F2-3) and dosed weekly with EFX or placebo. Following the 24-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 AK-US-001-0102 are supported by safety data from nonclinical studies and from the safety and efficacy data from the Phase 2a study.

NASH with fibrosis is a condition with a high risk of progression to cirrhosis which may lead to end-stage liver disease and increase the 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 this Phase 2b study. Efficacy will be determined by changes in liver histology. In addition, 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.

However, 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, this study will also incorporate non-invasive endpoints for the assessment of fibrosis including serum markers (e.g., ELF™ test, NIS-4, and pro-C3) and imaging (e.g., liver stiffness by FibroScan® and MRI-PDFF). A key exploratory objective of the study is [redacted]

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 resolution of steatohepatitis and crucially 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. 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 (≤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 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 adverse events than the 70mg dose. A total of 7 subjects discontinued from study drug due to TEAEs (4 in the 70 mg group, 0 in the 50 mg group, 2 in the 28 mg group, and 1 on placebo).

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. EFX exposures in non-cirrhotic subjects 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}.

Taking all the data into consideration, this study in NASH will evaluate the 28 and 50 mg doses, combining the likely effective dose range with acceptable safety and tolerability.

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

2.1. Primary Objectives

The primary objective of this study is:

- To evaluate the effect of EFX compared to placebo on fibrosis regression in NASH subjects with stage 2 or 3 fibrosis.

2.2. Secondary Objectives

The secondary objectives of this study are:

- To evaluate the effect of EFX compared to placebo on achieving NASH resolution in subjects with stage 2 or 3 fibrosis.
- To evaluate the effect of EFX compared to placebo on MRI-PDFF based on hepatic fat fraction.
- 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 glycemic control.
- To evaluate the effect of EFX compared to placebo on weight change.
- To assess the safety, tolerability, and immunogenicity of EFX.

2.3. Exploratory Objectives

- [redacted]

3. INVESTIGATIONAL PLAN

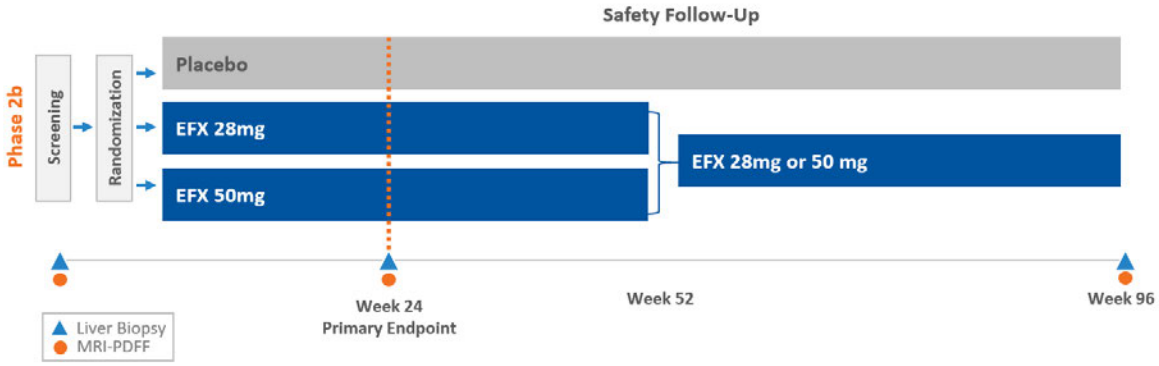
3.1. Overall Study Design and Plan

This is a Phase 2b, randomized, double-blind, placebo-controlled study evaluating the safety and efficacy of EFX in non-cirrhotic subjects with NASH. Subjects will be fibrosis stage 2 or 3 (F2-3) and dosed weekly with EFX or placebo.

Subjects meeting the study's eligibility criteria will be randomly assigned in a 1:1:1 ratio into 3 treatment groups as shown in Figure 2 below. Following 24 weeks of dosing, subjects will transition into the long-term safety follow-up portion of the study and will be followed until Week 96. If a subject refuses to complete the Week 24 liver biopsy, the subject will be discontinued from the study at Week 24. Only a 30-Day Follow-up visit is required following discontinuation of study drug. After all subjects have completed at least 52 weeks of treatment on their randomized dose, all subjects randomized to receive EFX will roll over into a single EFX dose arm (28 mg or 50 mg). The dose will be selected based on the Week 24 analysis and in alignment with regulatory authority.

Subjects on placebo will remain in the same treatment group for the duration of the study. After completing Week 96, subjects on placebo will be offered the option to receive EFX in a rollover study.

Figure 2. Study Schema



Randomization:

Randomization will be stratified by baseline type-2 diabetes status and fibrosis score. Study drug will be administered subcutaneously once weekly (QW). Eligible subjects will be randomized to one of three treatment groups with a ratio of 1:1:1.

Group A: Placebo

Group B: 28 mg EFX

Group C: 50 mg EFX

3.2. Study Duration and Number of Centers

Participation in the study can last up to 108 weeks, which includes 8 weeks for screening, 24 weeks to the primary endpoint, long-term follow-up for up to 96 weeks, and 30 days of follow-up.

This study will be conducted at approximately 55 sites in North America. Additional sites may be added, depending on subject accrual rates.

3.3. Early Termination

If discontinuation of study drug occurs, the subject should return to the clinic within 5 days to perform the Early Termination (ET) assessments.

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 has been completed.

The ET assessments are outlined in the Schedule of Assessments (Appendix A - B).

3.4. 30-Day Follow-up

The 30-Day Follow-up visit should be completed 30 days following the last dose of study drug received.

The 30-Day Follow-up assessments are outlined in the Schedule of Assessments (Appendix A - B).

3.5. Post Study Care

Subjects randomized to the placebo treatment arm who complete Week 96 will be offered the option to receive EFX in a rollover study.

4. STUDY POPULATION SELECTION

4.1. Study Population

The study will randomize approximately [redacted] subjects with biopsy proven NASH with fibrosis stage 2 or 3.

4.2. Inclusion Criteria

Subjects must meet all the following inclusion criteria to be eligible for study participation:

1. Males and non-pregnant, non-lactating females between 18 - 75 years of age inclusive, at the time of the screening visit.
2. Previous history or presence of 2 out of 4 components of metabolic syndrome (obesity, dyslipidemia, elevated blood pressure, elevated fasting glucose) or type 2 diabetes.
3. **Initial Screening Visit**: After a signed informed consent and confirmation of clinical risk profile associated with NASH, a screening lab panel and elastography will be measured for all subjects during an initial screening period:

    a. FibroScan® median liver stiffness > [redacted]
    b. CAP [redacted]
    Note: All subjects must complete a FibroScan® examination during the screening period. However, the median liver stiffness and CAP inclusion criterion do not apply to subjects with an eligible historical liver biopsy performed ≤ 180 days prior to randomization which confirmed fibrosis 1-3 and a NAS ≥ 4.

    If a historical value for FibroScan® is available in the past 3 months prior to the Screening Visit, then the FibroScan® does not need to be repeated.

    c. AST > 17 for females and > 20 for males;
    Note: The AST inclusion criterion does not apply to subjects with an eligible historical liver biopsy performed ≤ 180 days prior to randomization which confirmed fibrosis 2-3 and a NAS ≥ 4.

    d. Estimated glomerular filtration rate (eGFR) ≥ 60 mL/min, as calculated by the CKD-EPI equation;
    e. HbA1c ≤ 9.5% (or serum fructosamine ≤ 381 µmol if HbA1c is unavailable);
    f. INR ≤ 1.3, unless due to therapeutic anticoagulation;
    g. Direct bilirubin ≤ ULN;
    h. Total bilirubin ≤ upper limit of normal (ULN), unless due to an alternate etiology such as Gilbert's syndrome or hemolytic anemia;
    i. Creatinine kinase < 3 x ULN;
    j. Platelet count ≥ 140,000/µL;
    k. Triglyceride level ≤ 500 mg/dL;
    l. ALT < 5 x ULN;

m. AST < 5 x ULN;
n. ALP < 2 x ULN.

Note: Subjects meeting all of the above components will move into the next screening phase.

4. Documented historical stability (4 weeks to 6 months prior to screening) of ALT and AST levels showing no worsening at screening based on the following:

   a. If the historical and screening ALT and AST values are both ≤ 1.5 × the upper limit of normal (ULN), there is no limit to the difference between the values.
   b. If at least 1 of the historical values of ALT or AST is >1.5 × ULN and shows worsening at screening, the difference of ALT and AST values must be ≤ 50%.

   Note: Subjects without historical ALT and AST evaluations may have ALT and AST repeated (Pre-Baseline Visit) during the screening period at minimum 4 weeks apart to confirm a or b above;

   Subjects who are ineligible at the screening or pre-baseline visit may be re-screened or have an assessment repeated once if there is reasonable belief that the exclusionary result was obtained in error or is transient upon approval of the Medical Monitor.

5. MRI-PDFF [redacted]
   Note: All subjects must complete an MRI-PDFF examination during the screening period. However, the MRI-PDFF inclusion criterion does not apply to subjects with an eligible historical liver biopsy performed ≤ 180 days prior to randomization which confirmed fibrosis 2-3 and a NAS ≥ 4.

6. Biopsy-proven NASH. Must have had a liver biopsy obtained ≤ 180 days prior to randomization with fibrosis stage 2 to 3 and a non-alcoholic fatty liver disease (NAFLD) activity score (NAS) of ≥ 4 with at least a score of 1 in each of the following NAS components:

   a. Steatosis (scored 0 to 3),
   b. Ballooning degeneration (scored 0 to 2), and
   c. Lobular inflammation (scored 0 to 3)

7. Subjects on Vitamin E ≥ 400 IU/day, thiazolidinediones (including, but not limited to, pioglitazone, rosiglitazone, and lobeglitazone), GLP-1 agonists, or SGLT2 inhibitors must be on a stable dose (defined as no significant change in prescription efficacy, initiation of medication, or medication discontinuation) for at least 3 months prior to the diagnostic liver biopsy through randomization. A switch from one drug to another in the same class should be discussed with the Medical Monitor to confirm eligibility.
8. Subjects on antidiabetic, weight loss, or lipid-modifying medication(s) must be on stable dose(s) for at least 3 months prior to the diagnostic liver biopsy through randomization.

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 pregnancy test at screening and 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

Subjects meeting any of the following criteria are not eligible for study participation:

1. Weight loss > 5% in the 3 months prior to screening until randomization or from the time of the diagnostic liver biopsy until randomization, whichever is longer.
2. Presence of cirrhosis on liver biopsy (stage 4 fibrosis).
3. Type 1 diabetes.
4. Uncontrolled Type 2 diabetes defined as:

    a. Insulin dose adjustment >35% within 30 days prior to screening through randomization,
    b. Symptoms of the following within 3 months prior to screening: acutely decompensated blood glucose control (e.g., thirst, polyuria, weight loss), or a history of diabetic ketoacidosis, or a history of hyperglycemic hyperosmolar state

5. Hypoglycemia unawareness, hospitalization due to hypoglycemia, or history of severe hypoglycemia (hypoglycemia requiring outside assistance to regain normal neurologic status) within 3 months prior to screening.
6. Subjects with osteoporosis, defined as a T-score of -2.5 or lower at screening.
7. Poorly controlled hypertension (systolic blood pressure > 160 mm Hg, or diastolic blood pressure > 100 mm Hg).
8. Any prior history of decompensated liver disease including ascites, hepatic encephalopathy (HE), or variceal bleeding.
9. History of pancreatitis.
   Note: Subjects with a history of gallstone pancreatitis and subsequent cholecystectomy may be allowed to participate upon approval by the Medical Monitor.
10. Chronic hepatitis B virus (HBV) infection (hepatitis B surface antigen [HBsAg]) positive or acute hepatitis A infection (hepatitis A IgM antibody positive).
11. Chronic hepatitis C virus (HCV) infection (HCV antibody [Ab] and HCV ribonucleic acid [RNA] positive). Subjects cured of HCV infection < 2 years prior (based on date of RNA polymerase chain reaction [PCR] negative confirmation following conclusion of treatment) to the screening visit are not eligible.
12. Prior (< 2 years prior to screening) or planned (during the study period) bariatric surgery (e.g., gastroplasty, roux-en-Y gastric bypass). Surgery failure or reversal or removal of intragastric balloon > 2 years prior to screening would be acceptable.

13. Other causes of liver disease based on medical history and/or centralized review of liver histology, 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 requiring treatment.
14. History of liver transplantation.
15. Current or prior history of hepatocellular carcinoma (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 average of >20 g/day in female subjects and >30 g/day in male subjects.

17. Human immunodeficiency virus (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 the screening electrocardiogram (ECG) assessment.
19. Myocardial infarction, unstable angina, percutaneous coronary intervention, coronary artery bypass graft, or stroke within 3 months prior to screening.
20. Life expectancy less than 2 years.
21. Use of any investigational medication within 30 days or within 5 half-lives prior to screening or concurrent participation in another therapeutic clinical study. Participation in an experimental vaccine trial (e.g., COVID-19 or other) may be acceptable upon approval by the medical monitor.
22. Subjects with a history of (within 12 months prior to screening) or current use of prescription drugs associated with liver steatosis (e.g. methotrexate, amiodarone, high-dose estrogen, tamoxifen, systemic steroids, anabolic steroids, valproic acid). Short courses of systemic corticosteroids (less than two weeks) and 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.
23. Positive urine drug screen for amphetamines, cocaine or opiates (e.g., heroin, morphine) at screening. Subjects on stable methadone or buprenorphine maintenance treatment for at least 6 months prior to screening may be included in the study. 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.
24. Unable to safely undergo a liver biopsy.
25. Subjects who have contraindications to MR imaging (e.g., unmanageable claustrophobia, certain metal implants, or unable to fit within MR scanner due to girth).
26. Presence of any laboratory abnormality or significant systemic or major illnesses (other than liver disease) that, in the opinion of the Investigator, compromise 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 six months of screening.

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

5. INVESTIGATIONAL MEDICINAL PRODUCTS (IMP)

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. Subjects and all personnel directly involved in the conduct of the study will be blinded to treatment assignments. Investigational medicinal products 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 for that subject. When possible, Akero recommends but does not require that the Investigator contact the CRO 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 CRO medical monitor promptly in case of any treatment unblinding, 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 study treatment discontinued. All subjects will be followed until their 30-Day Follow-up visit unless consent to do so is specifically withdrawn by the subject.

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

5.2. Investigational Medicinal Product(s)

EFX is provided in 2 mL sterile vials filled with a 1.1 mL volume at strengths of 28 or 50 mg/mL. EFX will be presented as a sterile, colorless to slightly yellow and preservative-free frozen liquid.

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

After all subjects have completed at least 52 weeks of treatment on their randomized dose, all subjects randomized to receive EFX will roll over into a single EFX dose arm (28 mg or 50 mg). The dose will be selected based on the Week 24 analysis and in alignment with regulatory authority.

Subjects on placebo will remain in the same treatment group for the duration of the study. After completing Week 96, subjects on placebo will be offered the option to receive EFX in a rollover study.

Refer to the Pharmacy Manual for further instructions on EFX/placebo administration.

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

5.2.1. Storage and Handling

EFX / placebo vials will arrive frozen in individual cartons and must remain in their individual cartons and be immediately placed in a freezer maintained at ≤ -20°C until planned use. The EFX / 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. The set point is a single temperature and should remain constant. Do not refreeze EFX / placebo after it has been thawed.

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

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.

Records of the actual storage conditions during the period of the study must be maintained (e.g., records of the date and time and initials of person checking, and the "working day" temperatures of the refrigerator and freezer used for storage of study supplies, continuous temperature recordings, or regularly maintained temperature alarm systems used in conjunction with temperature recording).

The CRO must be notified if any IMP undergoes temperature excursions or if the IMP is damaged. Impacted IMP supply should be quarantined and should not be utilized until and unless Akero personnel have advised that it is acceptable to do so.

5.2.2. Dosage and Administration

Detailed information regarding the preparation and administration of EFX or placebo for this study is contained in the EFX Pharmacy Manual. Investigational medicinal product will be administered by subcutaneous injection once weekly as follows:

- Placebo QW (n = 40)
- 28 mg EFX QW (n = 40)
- 50 mg EFX QW (n = 40)

The IMP will be administered by a weekly 1.0 mL subcutaneous injection into the abdomen by a qualified staff member at the clinic or utilizing in-home nursing. The date and time of injection will be recorded. 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 antihistamine prior to injection.

If subjects are unable to attend study visits and/or study centers are unable to accommodate on-site visits due to pandemics, natural disasters, or other major disruptions procedures will be put in place to allow for direct-to-subject drug shipments for utilization of in-home nursing, as necessary.

5.3. Prior and Concomitant Medications

All concomitant medications will be recorded in the source documents and eCRFs. This includes concomitant medications taken within 30 days prior to screening and any taken during the study through end of the study.

Use of any investigational medication within 30 days or within 5 half-lives prior to screening and throughout the study is prohibited.

Subjects with a history of (within 12 months prior to screening) or current use of prescription drugs associated with liver steatosis (e.g. methotrexate, amiodarone, high-dose estrogen, tamoxifen, systemic steroids, anabolic steroids, valproic acid) should be excluded.

Examples of representative medications that are prohibited or which should be used with caution are listed below in Table 3 and Table 4.

Table 3. Prohibited Concomitant Medications

Drug Class	Agents Disallowed	Guidance
Select chronic immunosuppressants	Chronic systemic[a] corticosteroids	Short courses of systemic corticosteroids (less than two weeks) may be allowed prior to screening 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 12 months prior to screening until 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 > 10mg/day for > 2 weeks is not allowed.

Table 4. Conditionally Allowed Medications

Drug Class	Conditionally Allowed Medications	Guidance
Antioxidants	Vitamin E[a]	Doses >400 IU/day must be stable for at least 3 months prior to the diagnostic liver biopsy.
Anti-Hyperglycemic Therapy	Thiazolidinediones (including, but not limited to, pioglitazone, rosiglitazone,	Must be on a stable dose for at least 3 months prior to the diagnostic liver

Drug Class	Conditionally Allowed Medications	Guidance
	and lobeglitazone), GLP-1 agonists, or SGLT2 inhibitors[a]	biopsy through randomization. If possible, the dose of this medication should remain stable through the end of treatment.
	Insulin	Must be on a stable dose(s) for at least 3 months prior to the diagnostic liver biopsy through randomization. If possible, the dose of this medication should remain stable through the end of treatment. EFX is expected to improve insulin sensitivity, 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) for metformin are allowed in the 3 months prior to diagnostic liver biopsy. If possible, the dose of this medication should remain stable afterwards through the end of treatment.
	Sulfonylureas or other antidiabetic medication(s)	Must be on a stable dose(s) for at least 3 months prior to the diagnostic liver biopsy through randomization. If possible, the dose of this medication should remain stable through the end of treatment. EFX is expected to improve insulin sensitivity, 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)	Must be on a stable dose(s) for at least 3 months prior to the diagnostic liver biopsy through randomization. If possible, the dose of this medication should remain stable through the end of treatment.

a A stable dose is defined as no changes in prescribed dose, new medications, or discontinuation

5.4. Investigational Medicinal Product Accountability

The Sponsor or designee will supply the Investigational Medicinal Product (IMP). 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 IMP.

5.4.1. Investigational Medicinal Product 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 IMP supplies. If the site has an appropriate Standard Operating Procedure (SOP) for drug destruction, the site may destroy used and unused IMP 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 Akero (or Akero's representative) for return of unused IMP supplies. A copy of the site's SOP will be obtained for central files. Where possible, IMP 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 Akero. If IMP is destroyed on site, the Investigator must maintain accurate records for all IMP destroyed. Records must show the identification and quantity of each unit destroyed, the method of destruction, and person who disposed of the IMP. All IMP records must be maintained at the site and copies must be submitted to Akero during and at the conclusion of the study.

6. STUDY PROCEDURES

The following assessments will be conducted during screening and at selected time points specified in the Schedule of Assessments (Appendix A - B). Additional procedures deemed necessary as part of standard of care may be performed at the discretion of the Investigator. All missed visits and any performed procedures 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 can be implemented in case of crisis situations (e.g. COVID-19 pandemic, political strife, natural disaster) to protect subject safety, particularly in the event that a subject is unable to physically come to the site for an onsite visit and/or to be dispensed Investigational Medicinal Product (IMP). 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/IRB/ECs 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 and minimize risks to trial integrity, the sponsor determined that the following optional off-site study procedures can 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 they are 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 can 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 of 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

Before subjects commence any study-specific activities or procedures, the Sponsor requires a copy of the study site's written Institutional Review Board (IRB)/Independent Ethics Committee (IEC)/Ethics Committee (EC) approval of the protocol, informed consent form (ICF), and all other subject information and/or recruitment material, if applicable. 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 enters the screening period. Subjects will be screened up to 8 weeks before randomization to determine eligibility for participation in the study. The screening period may be extended under special circumstances with the explicit approval of the CRO Medical Monitor. Subjects who are ineligible at the screening visit may be re-screened or have an assessment repeated once if there is reasonable belief that the exclusionary result was obtained in error or is transient upon approval of the Medical Monitor.

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 decides that all of the eligibility criteria are met. The Investigator will document this decision and date and time 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, dates, and outcome of 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.

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. Clinically relevant physical exam findings prior to the first dose of study drug will be recorded on the medical history eCRF page 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.

Physical assessments required to calculate Child-Pugh Score (CP Score) are to be completed as part of the physical examination as indicated in the Schedule of Assessments.

6.5. Vital Signs, Weight, Hip and Waist Circumference

The following vital sign measurements will be performed as outlined in the schedule of assessments: systolic and diastolic blood pressure, pulse, respiration rate, temperature, height, weight, hip and waist circumference.

The subject must be in seated or in a semi-recumbent position in a rested and calm state for at least 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. Triplicate blood pressure (to be measured in same arm with at least two minutes rest between BP measurements) and heart rate to be collected at baseline and at the timepoints indicated in the schedule of assessments.

Height will be measured without shoes at screening.

Weight will be obtained at screening and selected time points thereafter. On dosing days, weight will be collected pre-dose. Weight should be obtained using a calibrated scale. The subject should be weighed in consistent clothing.

Waist circumference should be measured by placing the tape measure around the waist just above the top of the hip bone. Ensure it is snug against the skin, but not tight.

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

Weight, 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 the measurement.

6.6. 12-Lead Electrocardiography

The subject must be in a seated, semi-recumbent, or supine position in a rested and calm state for at least 10 minutes before ECG assessment is conducted. Each 12-lead ECG should be performed prior to blood draws, dosing (if applicable), or other invasive procedures. Each ECG must capture QRS, QT, QT interval corrected for heart rate (QTcF), RR, and PR intervals and be documented on the ECG eCRF.

The Investigator or designated study site physician will review, sign, and date all ECGs. 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.

6.7. Transient Elastography (FibroScan®)

Transient elastography will be performed using FibroScan® and 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 FibroScan® examinations.

At least 2-3 hours fasting is required prior to all elastography assessments.

Screening: All subjects much complete a FibroScan® examination during the screening period. However, the median liver stiffness and CAP inclusion criterion do not apply to subjects with an eligible historical liver biopsy performed ≤ 180 days prior to randomization which confirmed fibrosis 2-3 and a NAS ≥ 4.

If a historical value for liver stiffness based on transient elastography is available in the past 3 months prior to the Screening Visit, then the FibroScan® examination does not need to be repeated. The results of such a previous scan are to be recorded in the eCRF.

6.8. Liver Biopsy

All reasonable attempts should be made to acquire a needle core liver biopsy specimen of at [redacted] It is recommended that a [redacted] needle is used to collect the tissue sample. It is recommended that the subject should remain under observation consistent with the standard of care of that institution after the biopsy procedure. A liver biopsy must be obtained at screening to provide the liver tissue for central reading. A historical biopsy that meets eligibility criteria may be accepted as the screening biopsy if the sample is deemed acceptable for interpretation by the central readers. The historical sample must have been originally obtained ≤ 180 days prior to the Randomization date and should align with Section 5.3 Prior and Concomitant Medication guidance.

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 two, independent, trained pathologists. Both pathologists will read the same slides from all screening biopsies 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 two 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 24 liver biopsy, the subject will be discontinued from the study at Week 24. Only a 30-Day Follow-up visit is required following discontinuation of study drug.

6.9. MRI-PDFF

The degree of steatosis will be measured by MRI-PDFF. Subjects who have contraindications to MR imaging as outlined in the exclusion criteria should be excluded from the study. At least 4 hours fasting is required prior to all MR assessments.

The MRI-PDFF images will be analyzed by a blinded central reader. Please refer to the Imaging Manual for MRI-PDFF imaging guidelines.

Screening: All subjects must complete an MRI-PDFF examination during the screening period. However, the MRI-PDFF inclusion criterion does not apply to subjects with an eligible historical liver biopsy performed ≤ 180 days prior to randomization which confirmed fibrosis 2-3 and a NAS ≥ 4.

Rescreening: If a subject is rescreened with Medical Monitor approval, qualifying MRI-PDFF results from the subject's initial screening do not need to be resubmitted for analysis to the blinded central reader if the scan is obtained ≤ 4 months prior to randomization.

6.10. DXA Scan

A DXA scan should include lumbar spine, femoral neck, and total hip with subjects in the standard (supine) position.

Rescreening: If a subject is rescreened with Medical Monitor approval, qualifying DXA scan results from the subject's initial screening do not need to be resubmitted for analysis to the blinded central reader if the scan is obtained ≤ 6 months prior to randomization.

Please refer to the Imaging Manual for DXA imaging guidelines.

6.11. Chronic Liver Disease Questionnaire-Nonalcoholic Steatohepatitis (CLDQ-NASH)

The CLDQ-NASH asks questions related to liver disease and specifically NASH, to measure health related quality of life in subjects with chronic liver disease. 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 Baseline/Day 1, the questionnaire should not be collected at the remaining timepoints.

6.12. Lifestyle Guidance

All subjects will receive counseling regarding lifestyle including the maintenance of a healthy diet and physical activity at the Screening visit. 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.

6.13. Clinical Laboratory Tests

6.13.1. Laboratory Parameters

Blood samples will be collected according to the Schedule of Assessments (Appendix A - B) and below. Samples will be sent to the central laboratory for analysis and reporting. 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 of at least 8 or 12 hours.

Subjects will be in a seated, semi-recumbent, or supine position during blood collection. Clinical laboratory tests will include the following:

Table 5. Clinical Laboratory Assessments

Chemistry	Hematology/Coagulation	Urine/Miscellaneous	Biomarkers
Sodium Potassium Chloride Bicarbonate Calcium Magnesium Phosphorus Glucose[b] BUN Creatinine Total protein Albumin Uric acid LDH Total (TBL) and direct bilirubin Alkaline phosphatase (ALP) ALT (SGPT) AST (SGOT) GGT CK hsCRP Amylase Lipase[c] 3 Hydroxybutyrate HbA1c **Lipoproteins** Lp (a) NMR LipoProfile[b] (includes Total Cholesterol, HDL-C, LDL-C, LDL-P, Triglycerides)	**Hematology** RBC count Hemoglobin (Hgb) Hematocrit (Hct) Platelet count WBC count with differential: neutrophils, lymphocytes, monocytes, eosinophils, and basophils ANC **Coagulation** PT aPTT INR PAI-1 Fibrinogen **Genetic Substudy** [redacted]	**Urine** Drug screening: amphetamines, cocaine and opiates (i.e., heroin, morphine) **Miscellaneous** Screening Serology Tests HBsAg HBcAb HBV DNA Hep A IgM Anti-HCV antibody HCV RNA HIV Endogenous FGF21[b] Pregnancy test[a] Pharmacokinetics (PK) Anti EFX antibody (ADA) Neutralizing Anti EFX antibody (NAB)	**Fibrosis Biomarkers** ELF Pro-C3 C3M NIS-4[d] **Bone Biomarkers** P1NP CTX-1[b] **Lipid Metabolism Biomarkers** ApoB & ApoC3 **Insulin Sensitivity & Glycemic Control Biomarkers** Insulin Adiponectin C-Peptide HOMA-IR[b]

ADA = anti-drug antibodies; ALT = alanine aminotransferase; ANC = absolute neutrophil count; Apo = Apolipoprotein; AST = aspartate aminotransferase; aPTT = activated partial thromboplastin time; BUN = blood urea nitrogen; ELF = Enhanced Liver Fibrosis panel; GGT = gamma-glutamyl transferase; HbA1c = Glycated Hemoglobin A1c; HBcAb = total hepatitis B core antibody; HBsAg = hepatitis B surface antigen; HBV DNA = hepatitis B virus deoxyribonucleic acid; HCV = hepatitis C virus; HDL-C = high density lipoprotein cholesterol; HIV = human immunodeficiency virus; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; hsCRP = high sensitivity C-Reactive Protein; INR = International Normalized Ratio; LDH = lactate dehydrogenase; LDL-C = low density lipoprotein cholesterol; LDL-P = low density lipoprotein particle; Lp = Lipoprotein; L&HMW = low and high molecular weight; NMR = nuclear magnetic resonance; PAI-1 = Plasminogen Activator Inhibitor-1; Pro-C3 = N-terminal Type III Collagen Propeptide; PT = prothrombin time;

RBC = red blood cell; RNA = ribonucleic acid; TBL, total bilirubin; WBC = white blood cell

[a] For female subjects of childbearing potential only. Serum pregnancy test at screening and urine pregnancy test at other time points.

[b] Samples to be collected in the morning following an overnight fast of at least 8 hours.
[c] Reflex lipase testing is performed in subjects with total amylase > 1.5 ULN.
[d] Samples to be collected in the morning following an overnight fast of at least 12 hours

6.14. Sample Collection, Storage, and Shipping

Blood and urine samples from screening through ET or Follow-up visits will be collected and submitted to the central laboratory for analysis. Validated assays will be used for analysis when appropriate.

6.15. Pharmacokinetic Assessments

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

6.15.1 [redacted]

[redacted]

6.16. Anti-Drug Antibody and Neutralizing Antibody Sampling

Samples will be obtained for detection of anti-drug antibodies (ADA) against EFX as outlined in the Schedule of Assessments (Appendix A - B).

In the event of a positive anti-EFX antibody detection, a neutralizing ADA assay will be run for the sample timepoint 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 is to occur once the site has been notified of the positive result and approximately every 3 months 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 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.

6.17. Blood Storage

A portion of the blood samples drawn at study visits 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 Akero for a period of up to 15 years or per local guidelines.

6.18. 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 5. 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/EC approved ICF and applicable laws (e.g., health authority requirements).

6.19. Subject Retention

Given the burden of study assessments, long study duration, and potential commercial availability of treatments for subjects with NASH, maintaining subject retention in a long-term, placebo-controlled clinical outcomes study could be challenging. The objective of the subject retention plan is to describe the activities and tactics that can 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

Our aim will be to provide the sites with the support and tools to encourage behaviors that optimize subject engagement, retention and compliance:

- CRO staff and Akero staff will maintain regular contact (i.e. telephone calls or in-person as available) with the sites to specifically discuss retention and compliance at that site and to exchange and share experience 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.
- Site support materials will be provided to motivate and assist sites in engaging in subject retention activities.
- CRO staff and Akero staff will continue to reinforce key behaviors with site staff such as: encouraging staff to continue building subject relationship, conducting regular check-in phone calls or video-capable telehealth engagements, office visit reminder phone calls and ensuring the Principal Investigator is present at study visits, talking directly to the subject and re-enforcing the importance of their study participation.
- Provide support to minimize lost to follow-up through use of a third-party investigative service 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 & Retention Plan.

6.20. 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 6.24 for discontinuation.
- Subject experiences a Grade IV Common Terminology Criteria for Adverse Events (CTCAE) or meets the criteria for Drug Induced Liver Injury described in Section 6.26.
  - 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 6.26.3, 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 24 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 Akero, a regulatory agency, or an IRB/IEC/EC.

6.21. 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.

6.22. 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. AEs 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. AEs that occur after the first dose of EFX or during the study treatment and safety follow up periods will be documented on the AE eCRF. The Investigator will assess the AE severity and relationship of the AE to study drug. The Investigator will treat the subject as medically required.

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

From the time of obtaining informed consent until 30 days after the last administration of study drug, all SAEs and non-serious AEs related to protocol-mandated procedures will be recorded on the SAE/AE eCRF. All other untoward medical occurrences observed during screening, including exacerbation or changes in medical history, will be captured on the medical history eCRF. Details on recording and reporting AEs are provided below.

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.

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, until 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.

6.22.1. Severity

All AEs, both serious and non-serious, will be assessed for severity using the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. The CTCAE scale includes unique clinical descriptions of AEs that are categorized by anatomy and/or pathophysiology and is provided in the Site Operations Manual.

The CTCAE scale displays Grades 1 through 5 with unique clinical descriptions of severity for each AE (including abnormal laboratory values) based on this general guideline provided in the CTCAE scale. For any AEs not covered by CTCAE the conventional definition of severity will be used.

- Grade 1 (Mild) AE: minor; no specific medical intervention; marginal clinical relevance
- Grade 2 (Moderate) AE: minimal intervention; local intervention; non-invasive intervention
- Grade 3 (Severe) AE: significant symptoms requiring hospitalization or invasive intervention
- Grade 4 (Life-threatening or disabling) AE: Complicated by acute, life-threatening complications; need for intensive care or emergent invasive procedure
- Grade 5 AE: Fatal

6.22.2. 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 a SAE, 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.

6.22.3. 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 adverse event or lab abnormality (confirmed by repeat testing) deemed related to EFX.

- Two or more subjects develop the same treatment-emergent grade 4 or higher adverse event 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 adverse event 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 adverse events 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.

6.22.4. 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. AEs associated with overdose should be reported on relevant AE/SAE sections in the eCRF.

6.22.5. Serious Adverse Events

6.22.5.1. Definition

A 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.

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.

6.22.6. Reporting Adverse Events

6.22.6.1. 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 reported using 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 through the last day on the study participation 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.

6.22.6.2. Reporting Procedures for Serious Adverse Events

The Investigator is responsible for ensuring that all SAEs observed by the Investigator 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:

<u>Initial Reports</u>

- All SAEs occurring after the ICF has been signed or during the study treatment and safety follow up periods must be reported to Labcorp 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 Labcorp Clinical Safety or the Sponsor/designee.

- To report the SAE, complete the SAE form, indicating SAE criteria has been met, electronically in the electronic data capture (EDC) system for the study. If the event meets serious criteria and it is not possible to access the EDC system, fax/email the completed paper SAE form to Labcorp Safety at SAEIntake@Labcorp.com or 1-888-887-8097 within 24 hours of awareness.
  NOTE: any reports faxed to 1-888-887-8097 will be routed to SAEIntake@Labcorp.com. When the EDC system becomes available, the SAE information must be entered within 24 hours.

  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 electronically in the EDC system for the study, and submit any supporting documentation (e.g., subject discharge summary or autopsy reports) to Labcorp 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 AE form and the concomitant medication eCRF.

Follow-up of SAEs will continue through the last day on the study 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 safety follow up period defined in the protocol, 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.

A pregnancy is not considered to be an AE or SAE; however, it must be reported to Labcorp Clinical Safety within 24 hours of knowledge of the event. Labcorp 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 Labcorp Clinical Safety.

If the female partner of a male subject becomes pregnant while the subject is receiving study drug or within the safety follow up period defined in the protocol, the Investigator should notify Labcorp 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 Labcorp 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.

Safety Contact Information:
Labcorp Clinical Safety
Facsimile: United States 1-888-887-8097 (Global fax numbers located in Study Binder)
E-mail: SAEIntake@Labcorp.com

Some SAEs will qualify for reporting to the Food and Drug Administration (FDA) as applicable via the MedWatch/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 according to local regulations and Good Clinical Practice (GCP).

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

The Investigator will notify the appropriate IRB/IEC/EC 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 Investigator's Brochure) to the IRB/IEC/EC and for retaining a copy in the study files.

6.23. Adverse Events of Special Interest

The Investigator will monitor each subject for clinical and laboratory-evidence for predefined AEs of special interest (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 6.26.

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 adverse events while on study should be managed according to local standard of care including concomitant medications to manage symptoms.

6.24. 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, urinalysis) independent of the underlying medical condition that require medical or surgical intervention or lead to investigational medicinal product 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., electrocardiogram, 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 6.22. 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 Version 5.0. 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.

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

Grade 1 and 2 Laboratory Abnormality or Clinical Event
Continue investigational medicinal product at the discretion of the Investigator.

Grade 3 Laboratory Abnormality or Clinical Event

- For Grade 3 clinically significant laboratory abnormality or clinical event, investigational medicinal product may be continued if the event is considered to be unrelated to investigational medicinal product.

- For a Grade 3 clinical event, or clinically significant laboratory abnormality confirmed by repeat testing, that is considered to be related to investigational medicinal product, investigational medicinal product should be withheld until the toxicity returns to ≤ Grade 2.

- If a laboratory abnormality recurs to ≥ Grade 3 following re-challenge with investigational medicinal product and is considered related to investigational medicinal product, then investigational medicinal product should be permanently discontinued, and the subject managed according to local practice. Recurrence of laboratory abnormalities considered unrelated to investigational medicinal product 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 investigational medicinal product 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 a laboratory abnormality recurs to ≥ Grade 4 following re-challenge with investigational medicinal product and is considered related to investigational medicinal product, then investigational medicinal product should be permanently discontinued, and the subject managed according to local practice. Recurrence of laboratory abnormalities considered unrelated to investigational medicinal product 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 6.20, investigational medicinal product should be discontinued permanently.

6.25. 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.

6.25.1. Hepatic Events Adjudication Committee

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

1) Progression to cirrhosis as defined by a liver biopsy showing F4 fibrosis according to the NASH CRN classification, as assessed by the central reader.

2) Events of hepatic decompensation including:

a) Clinically apparent ascites requiring treatment

b) HE 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

3) 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 4 weeks apart

4) All-cause mortality

Each of the clinical events (except histologic progression to cirrhosis, 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.

If there is clinical evidence that a subject has progressed to cirrhosis (e.g., based on the presence of new esophageal varices, changes in biomarkers [including, but not limited to, low serum albumin, high serum bilirubin, a low platelet count, prolonged INR, or elevated liver stiffness], or development of other clinical signs or symptoms of cirrhosis), the subject should undergo repeat liver biopsy for confirmation of progression to cirrhosis (F4 fibrosis as assessed by the central reader according to the NASH CRN classification) at the discretion of the principal investigator (PI).

Once the clinical event (except histological progression to cirrhosis, all-cause mortality and liver transplantation) is confirmed by the Hepatic Events Adjudication Committee, the subject will discontinue from the study. Subjects who experience histological progression to cirrhosis, all-cause mortality and liver transplantation will be discontinued from the study, but do not require confirmation from the adjudication committee prior to discontinuation.

6.25.2. Cardiovascular Events Adjudication Committee

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 PI.

6.26. Observation for Drug Induced Liver Injury (DILI)

Throughout the study, all TEAEs, clinical assessments, and clinical laboratory parameters, and the criteria for potential drug-induced liver injury will be closely monitored with subjects entering close observation as detailed in this protocol.

6.26.1. 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 - 5 days from receipt of results.
    - If there are persistent elevations (ALT > 5 × ULN or TBL > 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 investigational medicinal product should be considered.
- Study drug should be discontinued, and the subject should be followed until resolution of signs or symptoms, in the following situations:
    - ALT >8 × ULN
    - ALT >5 × ULN and (TBL >2 × ULN)
    - 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 PI 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 investigational medicinal product may be considered after consultation with the Medical Monitor and DMC assessment.

6.26.2. 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 - 5 days.

- 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.

- Discontinue the study drug if any of the following occur:
  - ALT increases to >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 TBL to > 2 × ULN.
  - 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 investigational medicinal product may be considered after consultation with the CRO Medical Monitor and DMC assessment.

6.26.3. Close Observation for Suspected Drug-Induced Liver Injury

For all subjects, close observation for suspected drug-induced liver injury includes the following within 72 hours of suspected drug-induced liver injury:

- Repeating liver enzyme (ALT, AST, and alkaline phosphatase) 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.
- Obtaining a more detailed history of symptoms and prior or concurrent diseases.
- Obtaining a history of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
- Ruling out acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease.
- Obtaining a history of exposure to environmental chemical agents.
- Obtaining additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin).
- Considering gastroenterology or hepatology consultations.
- 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.

6.27. 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
- At the discretion of the Investigator and/or study 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

The primary endpoint is:

- Proportion of subjects who achieve improvement in liver fibrosis, defined as ≥ one stage (NASH CRN fibrosis score) and no worsening of steatohepatitis (defined as no increase in NAS for ballooning, inflammation, or steatosis) at Week 24.

7.1.1.2. Secondary Endpoints

The secondary endpoints are:

- Proportion of subjects who achieve resolution of steatohepatitis (defined as a NAS of 0-1 for inflammation, 0 for ballooning, and any value for steatosis) and no worsening of liver fibrosis as determined by the NASH CRN criteria at Week 24 and Week 96.
- Proportion of subjects who achieve improvement in liver fibrosis, defined as ≥ one stage (NASH CRN fibrosis score) and no worsening of steatohepatitis (defined as no increase in NAS for ballooning, inflammation, or steatosis) at Week 96.
- Proportion of subjects who achieve improvement in liver fibrosis, defined as ≥ one stage (NASH CRN fibrosis score) at Week 24 and Week 96.
- Change from baseline in hepatic fat fraction measured by MRI-PDFF at Week 24 and Week 96.
- Change from baseline of lipoproteins – triglycerides, Non-HDL-C, HDL-C and LDL-C.
- Change from baseline of markers of glycemic control – HbA1c, C-peptide, Adiponectin and HOMA-IR.
- Change from baseline in non-invasive markers of fibrosis – ELF, Pro-C3, C3M, NIS-4 and liver stiffness assessed by transient elastography (FibroScan®).
- Change from baseline of body weight.

7.1.1.3. Exploratory Efficacy Endpoints

The exploratory endpoints are:

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

7.1.2. Safety Endpoints

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

Safety analyses include: summaries of extent of exposure, AEs, clinical laboratory tests, endogenous FGF21, ECG, BMD, vital sign assessments, body weight, anti-drug antibody (ADA) and neutralizing antibody (NAB) assessments, and concomitant medication usage.

7.2. 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).

7.3. Analysis Sets

The study analysis sets are detailed below.

7.3.1. Full Analysis Set (FAS)

The primary analysis set for non-histological efficacy analyses will be the Full Analysis Set (FAS) which will include all subjects who were randomized into the study.

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.

7.3.2. 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.

7.3.3. Safety Set

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

7.3.3.1. 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 of EFX is available.

7.3.3.2. 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.

7.3.3.3. Liver Biopsy Analysis Set (LBAS)

The primary 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.

7.3.3.4. MRI-PDFF Analysis Set

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.

7.4. 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 that 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 is not dosed then they 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.

7.5. Estimands

In line with 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 matching placebo administered weekly.

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

The population of subjects targeted is adults with NASH with liver fibrosis as defined by the inclusion/exclusion criteria.

Intercurrent events are handled through a composite strategy (see Table 6).

The population-level summary of the treatment effect will be quantified by comparing observed responder proportions supported by the risk ratio of investigational product 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 Statistical Analysis Plan.

Table 6. Approach for Intercurrent Events for the Primary Endpoint

Intercurrent Event	Status
Start of additional therapy with potential to impact NASH condition (i.e. rescue medication)	Subject defined as a non-responder (even if a post-treatment biopsy is available)
MELD score ≥15	Subject defined as a non-responder (even if a post-treatment biopsy is available)
Hepatic decompensation event	
Unscheduled biopsy showing progression to F4	Subject will be counted as a non-responder
Bariatric surgery	Subject defined as a non-responder

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

7.6. 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, waist-to-hip ratio, randomization stratification groups, and other disease characteristic variables.

7.7. Efficacy Analysis

7.7.1. Primary Efficacy Analysis

The primary efficacy analysis will be conducted when all the planned subjects complete the 24-Week 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 between the EFX arms and the placebo arm, adjusting for stratification factors. The analysis will be performed using the LBAS. The point estimates and [redacted] confidence intervals for the differences in proportions will be calculated.

The Primary efficacy endpoint will be tested at Type I error rate of [redacted], two-sided without adjustment for multiplicity.

7.7.2. Secondary Efficacy Analysis

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 controlling for stratification factors will be used to evaluate the changes from baseline to Week 24 in hepatic fat fraction and liver stiffness between either EFX arm and the placebo arm in the Week 24 analysis.

A mixed-model repeated-measures (MMRM) will be used to evaluate the changes from baseline in hepatic fat fraction, triglycerides, Non-HDL-C, HDL-C, LDL-C, HbA1c, C-peptide, HOMA-IR, non-invasive fibrosis markers, liver stiffness, and body weight between the EFX arm and the placebo arm at scheduled visits, controlling for stratification factors.

Summaries and will be presented for changes from baseline in triglycerides, Non-HDL-C, HDL-C, LDL-C, HbA1c, C-peptide, HOMA-IR, and body weight at the collection timepoints defined in the Schedule of Assessments by treatment arm.

7.7.3. Exploratory Efficacy Analysis

The same efficacy analyses used for the secondary efficacy variables will be used for the exploratory efficacy variables.

7.8. 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.

7.8.1. Extent of Exposure

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

7.8.2. 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), Preferred Term (PT), and Lower-Level Term (LLT) will be attached to the clinical database. AE severity will be graded using the CTCAE.

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).

7.8.3. Laboratory Evaluations

Selected laboratory data will be summarized (n, mean, SD, 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. Grading of laboratory abnormalities for analysis purposes will be defined in the Statistical Analysis Plan.

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.

7.9. Pharmacokinetic Analysis

Plasma concentrations of EFX will be listed and summarized pre-dose at the timepoints listed in the Schedule of Assessments. Noncompartmental parameters will be listed and summarized descriptively.

7.10. Biomarker Analysis

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

Exploratory analyses may also be performed to evaluate the association of individual exploratory biomarkers or combination of biomarkers with clinical measurements and other risk factors.

Additional exploratory analyses that could enhance the understanding of the biological effects and the mechanism of action of EFX may be added in the Biomarker Analysis Plan if necessary.

7.11. 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 PD will be analyzed.

7.12. Sample Size

The primary endpoint is the percentage of subjects with improvement in liver fibrosis ≥ one stage and no worsening of steatohepatitis at Week 24. It is predicted [redacted] of subjects will reach this endpoint for the placebo group, based on published results. Based on phase 2a BALANCED study of EFX it is estimated that approximately [redacted] subjects of each EFX dose group will reach this endpoint. With two-sided Pearson chi-square test for proportion difference, using significance level of [redacted] completed subjects per group will provide at least [redacted] power. Factoring in dropouts, the study plans to randomize at least [redacted] subjects in total [redacted] subjects per group).

7.13. Data Monitoring Committee

An external DMC that consists of two hepatologists, one cardiologist and a statistician will review the progress of the study. The DMC will convene after 45 subjects (approximately 15 per treatment group) have completed the Week 12 assessments. The DMC will receive all reports of SAEs, potential DILI events for evaluation and convene as needed to monitor for safety and additional meetings will be scheduled as required. The DMC will provide recommendations to Akero 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 compared with the assumed prevalence rate per year.

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 6.26). 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 will be allowed to continue in the study according to the follow-up procedures outlined in Section 6.26.

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 Akero regarding future conduct of the study, including possible early study termination, Akero 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, International Council for Harmonisation (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 European Union Clinical Trials Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC.

The Investigator will ensure adherence to the basic principles of Good Clinical Practice, as outlined in 21 Code of Federal Regulations (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 subinvestigators will comply with 21 CFR, Part 54, 1998, providing documentation of their financial interest or arrangements with Akero, or proprietary interests in the investigational medicinal product under study. This documentation must be provided prior to the Investigator's (and any subinvestigator's) participation in the study. The Investigator and subinvestigator 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)/ Ethics Committee (EC) 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/EC. The Investigator will not begin any study subject activities until approval from the IRB/IEC/EC 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/EC any modifications made to the protocol or any accompanying material to be provided to the subject after initial IRB/IEC/EC 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/EC-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/EC, 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 IMP, 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 two categories: (1) Investigator's study file, and (2) subject clinical source documents.

The Investigator's study file will contain the protocol/amendments, CRF and query forms, IRB/IEC/EC 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, electrocardiograms, 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 informed consent form 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., United States, 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 electronic Case Report Forms 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/EC, 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/EC in accordance with local requirements and receive documented IRB/IEC/EC 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 Akero 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 appropriate regulatory authorities, IRBs, IECs, and ECs. 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

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10. APPENDICES

Appendix A. Schedule of Assessments – Screening to Week 24

Assessments [a]	Screening [t] (- 8 wks)	Pre-Baseline [b] (-4 wks)	Baseline / Day 1	Week 1	Weeks 2 & 3	Week 4	Week 8	Week 12	Week 16	Week 20	Weeks 5, 6, 7, 9, 10, 11, 13, 14, 15, 17, 18, 19, 21, 22, 23 [c]	Week 24	Early Term & 30-Day Follow-Up [r]
	Clinical Assessments												
Written Informed Consent	X												
Eligibility	X	X	X										
Medical History	X												
Physical Examination [d]	X		X	X		X	X	X	X	X		X	X
Child-Pugh Score [d]			X					X				X	X
Vital Signs [e]	X	X	X	X	X	X	X	X	X	X		X	X
Weight [f]	X	X	X	X	X	X	X	X	X	X		X	X
Height	X												
Hip and Waist Circumference [f]	X		X					X				X	X
12-lead ECG	X		X									X	X
FibroScan® [g]	X											X	X
MRI-PDFF	X											X	X[s]
DXA	X												
Liver Biopsy [a]	X											X	X[s]
CLDQ-NASH [h]			X					X				X	

Assessments [a]	Screening [t] (- 8 wks)	Pre-Baseline [b] (-4 wks)	Baseline / Day 1	Week 1	Weeks 2 & 3	Week 4	Week 8	Week 12	Week 16	Week 20	Weeks 5, 6, 7, 9, 10, 11, 13, 14, 15, 17, 18, 19, 21, 22, 23 [c]	Week 24	Early Term & 30-Day Follow-Up [r]
	Clinical Assessments												
Lifestyle Guidance [i]	X		X	X	X	X	X	X	X	X		X	
Administer Study Drug [j]			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
Adverse Events	X [k]	X	X	X	X	X	X	X	X	X	X	X	X
	Laboratory Assessments												
Chemistry, Hematology, Lipids, Coagulation [l]	X	X [m]	X	X		X	X	X	X	X		X	X
Serum & Plasma Storage Samples [n]			X	X		X	X	X	X	X		X	X
Urine drug screen	X												
Serologies [l]	X												
Fibrosis Biomarkers [l]			X					X				X	X
Other Biomarkers [l]			X			X		X				X	X
PK [o]			X			X	X		X			X	X
ADA and NAB [p]			X				X		X			X	X
Endogenous FGF21			X				X		X			X	X
[redacted]							[redacted]						
Pregnancy testing [q]	X		X			X	X	X	X	X		X	X

ADA = anti-drug antibodies; BP = blood pressure; ECG = electrocardiogram; HR = heart rate; PE = Physical Examination; PK = pharmacokinetic; NAB = neutralizing antibody

a All visits after Baseline (Day 1) can be performed within the following window: ± 3 days for weekly visits 1 - 24. Liver Biopsies should be obtained within (–) 2 weeks to (+) 4 weeks of the scheduled Week 24 visit date. MRI-PDFF to be obtained ± 5 days from the liver biopsy.

b If no historical ALT/AST measurements available, visit is required to confirm no worsening of ALT/AST.

c Subjects will have the option to conduct visit using in-home nursing.
d Complete PE including assessment at Screening, Baseline and Weeks 12 & 24, otherwise symptom-driven. Child-Pugh (CP) Score assessments should be completed as part of the physical exam at Baseline, Week 12, Week 24, and ET & 30-Day Follow-up.
e Vitals to include systolic and diastolic BP, pulse, respiration rate will be collected at each visit. Temperature will also be collected at Screening and Baseline only. Triplicate blood pressure (to be measured in same arm with at least two minutes rest between BP measurements) and heart rate to be collected at Baseline, Week 12 & Week 24 and single measurements to be collected at all other visits.
f Weight, 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 the measurement. On in clinic dosing days, weight will be collected pre-dose. Weight should be obtained using a calibrated scale. The subject should be weighed in consistent clothing.
g At least 2-3 hours fasting is required prior to all elastography assessments.
h If the CLDQ-NASH is missed at Baseline/Day 1, questionnaire should not be collected at the remaining timepoints. 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.
i All 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.
j Administration of study drug should occur following all other study procedures and sample collection for that visit.
k AE reporting during screening is limited to SAEs and AEs related to study procedures.
l Refer to Table of Clinical Laboratory Assessments. Samples to be collected in the morning following an overnight fast of at least 8 or 12 hours where indicated.
m Only confirmatory ALT/AST to be collected at the Pre-Baseline Visit to confirm eligibility if required.
n Serum & plasma storage samples banked for possible additional clinical testing.
o PK sampling will be obtained pre-dose.
p Samples will be obtained for detection of ADA against EFX and if required a neutralizing ADA assay will be run on the same sample.
q For female subjects of childbearing potential only. Serum pregnancy test at screening and urine pregnancy test at other time points.
r Early Termination visit should occur within 5 days of discontinuation of study drug. The 30-Day Follow-Up visit should occur 30 days following the last dose of study drug received.
s Liver Biopsy to be obtained at Early Termination only if the subject received at least 16 weeks of EFX/Placebo. MRI to be obtained ± 5 days from the liver biopsy.
t The screening period may be extended by 2 weeks under special circumstances with the explicit approval of the CRO Medical Monitor.

Appendix B. Schedule of Assessments – Long-Term Follow-Up

Assessments [a]	Weeks 28, 36, 44, 52	Week 32, 40	48	Weeks 56, 64, 80, 88	Week 72	Weeks 96	Early Term & 30-Day Follow-Up [l]
	Clinical Assessments						
Physical Examination	X	X	X [b]	X	X	X	X
Child Pugh Score [b]			X			X	X
Vital Signs [c]	X	X	X	X	X	X	X
Weight [d]	X	X	X	X	X	X	X
Hip and Waist Circumference [d]	X	X	X	X	X	X	X
12-lead ECG			X			X	X
FibroScan® [e]			X			X	
MRI-PDFF						X [m]	X [o]
DXA			X [p]			X	
Liver Biopsy						X [m]	X [o]
CLDQ-NASH [f]	X		X			X	
Lifestyle Guidance [g]	X	X	X	X	X	X	
Administer Study Drug [h]	X	X	X	X	X	X	
Concomitant Medications	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X
	Laboratory Assessments						
Chemistry, Hematology, Lipids, Coagulation [i]	X	X	X	X	X	X	X
Serum & Plasma Storage Samples [j]	X	X	X	X	X	X	X

Assessments [a]	Weeks 28, 36, 44, 52	Week 32, 40	48	Weeks 56, 64, 80, 88	Week 72	Weeks 96	Early Term & 30-Day Follow-Up [l]
Fibrosis Biomarkers [i]			X		X	X	X
Other Biomarkers [i]		X	X		X	X	X
PK		X	X	X	X	X	X
ADA and NAB [n]		X	X	X	X	X	X
Endogenous FGF21		X	X	X	X	X	X
Pregnancy testing [k]	X	X	X	X	X	X	X

ADA = anti-drug antibodies; BP = blood pressure; ECG = electrocardiogram; HR = heart rate; PE = Physical Examination; PK = pharmacokinetic; NAB = neutralizing antibody

a In-clinic visits will be conducted every 4 weeks ± 5 days from Week 28 to Week 56 and then every 8 weeks ± 7 days from Week 64 to Week 96.

b Complete PE including assessment at Week 48, otherwise symptom-driven. Child-Pugh (CP) Score assessments should be completed as part of the physical exam at Week 48, Week 96, and ET & 30-Day Follow-up.

c Vitals to include systolic and diastolic BP, pulse, respiration rate will be collected at each visit. Triplicate blood pressure (to be measured in same arm with at least two minutes rest between BP measurements) and heart rate to be collected at Week 48 and Week 96. Single measurements to be collected at all other visits.

d Weight, 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 the measurement. On dosing days, weight will be collected pre-dose. Weight should be obtained using a calibrated scale. The subject should be weighed in consistent clothing.

e At least 2-3 hours fasting is required prior to all elastography assessments.

f If the CLDQ-NASH is missed at Baseline/Day 1, questionnaire should not be collected at the remaining timepoints. 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.

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

h Administration of study drug should occur following all other study procedures and sample collection for that visit. Beginning at Week 25, weekly administration of study drug is required either on site or utilizing in-home nursing for the following visits: Weeks 25-27, 29-31, 33-35, 37-39, 41-43, 45-47, 49-51, 53-55, 57-63, 65-71, 73-79, 81-87, and 89-95. Administration of last dose of study drug will occur at Week 95.

i Refer to Table of Clinical Laboratory Assessments. Samples to be collected in the morning following an overnight fast of at least 8 or 12 hours where indicated.

j Serum & plasma storage samples banked for possible additional clinical testing.

k For female subjects of childbearing potential only. Urine pregnancy test at all time points.

l Early Termination visit should occur within 5 days of discontinuation of study drug. The 30-Day Follow-Up visit should occur 30 days following the last dose of study drug received.

m Additional biopsy required at Week 96. The liver biopsy should be obtained (–) 8 weeks to (+) 4 weeks from the scheduled visit date. MRI-PDFF to be obtained ± 5 days from the liver biopsy.

n Samples will be obtained for detection of ADA against EFX and if required a neutralizing ADA assay will be run on the same sample.

o Liver Biopsy may be required for adjudication of pre-specified liver-related outcomes or to be obtained at Early Termination only if the previous liver biopsy was obtained ≥ 12 months prior. MRI-PDFF to be performed if a liver biopsy is obtained, ± 5 days from the liver biopsy.
p DXA to be obtained ± 10 days of the Week 48 Visit.

Appendix C [redacted]

[redacted]

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 post-menopausal, unless permanently sterile or with medically documented ovarian failure.

Women are considered to be in a postmenopausal state when they are $\geq$ 54 years of age with cessation of previously occurring menses for $\geq$ 12 months without an alternative cause. In addition, women of any age with amenorrhea of $\geq$ 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 of 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.

EFX has not yet been studied in pregnant women.

Please refer to the latest version of the Investigator's Brochure 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. They must have a negative serum pregnancy test at screening and a negative pregnancy test on the Baseline/Day 1 visit prior to randomization. Pregnancy tests will be performed per Schedule of Assessments. Female subjects must agree to one of the following from screening until the 30-Day Follow-up Visit.

- 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 (IUD) with a failure rate of < 1% per year

  — Intrauterine hormone-releasing system (IUS) 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 procedure)

Should female subjects wish to use a hormonally based method, 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 three months prior to study dosing. 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: please 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 one 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 (LAM). 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 6.22.6.2.

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 three of the followings 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 three 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