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Rationale and design of a randomized, double-blind, placebo-controlled, parallel-group, investigator-initiated phase 2a study to investigate the efficacy and safety of elobixibat in combination with cholestyramine for nonalcoholic fatty liver disease

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Study dates: November 1, 2019 to December 31, 2020.

Abstract

Introduction: Nonalcoholic fatty liver disease (NAFLD) pathogenesis involves abnormal cholesterol metabolism and hepatic accumulation of toxic free-cholesterol. Elobixibat (EXB) inhibits the ileal bile acid (BA) transporter. EXB and cholestyramine (CTM) facilitate the removal of free cholesterol from the liver by diminishing BA recirculation to the liver, thereby stimulating novel BA synthesis from cholesterol. This randomized, double-blind, placebo-controlled, parallel-group, phase IIa study aims to provide a proof-of-concept assessment by evaluating the efficacy and safety of EXB in combination with CTM in patients with NAFLD.

Methods and analysis: A total of 100 adult patients with NAFLD according to low-density lipoprotein cholesterol (LDL-C) >120 mg/dL and ≥8% liver fat content on magnetic resonance imaging (MRI)-based proton density fat fraction (MRI-PDFF) that meet the inclusion/exclusion criteria will be enrolled. The patients will be randomly assigned to receive combination therapy of 10 mg EXB and 9 g CTM powder (4 g CTM), 10 mg EXB monotherapy, 9 g CTM powder monotherapy, or a placebo treatment (n=25 per group). Blood tests and MRIs will be performed 16 weeks following the therapy initiation. The primary study endpoint will be the absolute LDL-C level change at week 16 after treatment initiation. The secondary endpoint will include absolute changes in the liver fat fraction as measured by MRI-PDFF. This proof-of-concept study will determine whether the combination therapy of EXB and CTM is effective and safe for patients with NAFLD.

Ethics and dissemination: Ethics approval was obtained from the Ethics Committee of Yokohama City University Hospital prior to participant enrollment. The results of this study will be submitted for publication in international peer-reviewed journals and the key findings presented at international scientific conferences.

Trial registration: This trial is registered with ClinicalTrials.gov (number NCT04235205).

Protocol version: 1.3, February 20, 2020

Keywords: Nonalcoholic fatty liver disease, elobixibat, cholestyramine, combination therapy, low-density lipoprotein cholesterol, phase 2a

Strengths and limitations of this study

- The is the first proof-of-concept study to assess the efficacy and safety of the combination therapy consisting of elobixibat and cholestyramine in patients with nonalcoholic fatty liver disease.
- The trial is a randomized, double-blind, placebo-controlled, parallel-group, phase IIa study.
- We hypothesize that the combination therapy of elobixibat and cholestyramine will have higher efficacy and fewer side effects than either monotherapy.

- The primary study endpoint is the change in the absolute low-density lipoprotein cholesterol level. The secondary endpoint monitors the absolute changes in the liver fat fraction measured by magnetic resonance imaging proton density fat fraction.
- Our study also has limitations as follows: (1) performed within a single center; (2) a relatively small sample size; (3) a relatively short treatment duration; and (4) the lack of liver biopsy.

1. Introduction[1]

Nonalcoholic fatty liver disease (NAFLD) is a clinical condition that is detected by tissue or image analyses and diagnosed by excluding alcoholism and other liver diseases. NAFLD is the hepatic manifestation of a metabolic syndrome and is often associated with obesity, diabetes mellitus, dyslipidemia, hypertension, and other disorders. NAFLD prevalence increased worldwide, with an increase in Japan from 12.9% in 1994 to approximately 34.7% in 2000 [1]. NAFLD is classified as nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH), which includes inflammation and a progressive nature associated with liver cancer or cirrhosis developing in 10–20% of the patients [2]. Contributing NASH pathogenesis factors are abnormal cholesterol metabolism and free cholesterol accumulation in the liver, which is toxic to hepatocytes and leads to inflammation and fibrosis [3].

Bile acids (BAs) may be critical in NASH pathogenesis [4-6]. BAs act as signaling molecules involved in lipid, glucose, and energy homeostasis, whose metabolic pathways are linked to NAFLD/NASH and comorbidities, including metabolic syndrome, obesity, and diabetes. The BAs and free cholesterol as the precursor for synthesizing BAs can act as lipotoxic agents that drive inflammation and fibrosis. In previous studies, both serum and liver BA levels were elevated in NASH patients, and recent data suggested that the presence and severity of NASH are associated with specific changes in circulating BAs [6]. Therefore, targeting BA pathways may have therapeutic potential for patients with NAFLD.

Elobixibat (EXB) inhibits the ileal BA transporter (IBAT) and is approved for marketing as an oral treatment of chronic constipation [7]. The IBAT expressed primarily in the distal ileum is a key element in the enterohepatic circulation of BAs as it facilitates the highly efficient process of BA reabsorption. EXB is orally administered and acts locally in the gut, where it reversibly binds to IBAT to decrease the re-uptake of BAs to the liver. EXB at expected therapeutic doses has minimal systemic exposure. Pharmacodynamic assessment of EXB in a Japanese phase I study showed decreased serum LDL-C levels in the groups that received repeated doses of ≥5 mg [8]. In addition, it has been reported for an NAFLD mouse model that treatment with another IBAT inhibitor

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[1]**Abbreviations**

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FAS, full analysis set; γ-GTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MOA, mechanism of action; MRI-PDFF, magnetic resonance imaging-based proton density fat fraction; MRE, magnetic resonance elastography; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; POC, proof-of-concept; SAS, safety analysis set.

(IBATi) promotes BA biosynthesis from cholesterol, which accumulates in the liver of the mice receiving a high-fat diet [9]. Furthermore, converting the BA pool in the liver into hydrophobic BAs decreases hepatic cholesterol by farnesoid X receptor (FXR) activation and results in improved NAFLD status [9]. EXB is also expected to improve the NAFLD status as it has the same mechanism of action (MOA) and lowers LDL-C levels.

However, increased BAs in the large intestine may stimulate secretion of water and electrolytes in the intestinal tract and gastrointestinal movement, which induces BA diarrhea and promotes peristalsis [10, 11].

The BA sequestrant (BAS) cholestyramine (CTM) is a strong base anion exchange resin, and its drug product treats hypercholesteremia and eliminates active leflunomide metabolites from the body. CTM inhibits the absorption of exogenous cholesterol by binding to BA in the intestinal tract, which increases feces excretion. CTM also promotes cholesterol catabolism to BA in the liver to compensate for decreased BA levels due to increased excretion. This ultimately decreases LDL-C levels. These effects are expected to promote hepatic lipid metabolism. In a Japanese clinical study, colesevelam, another BAS, improved fatty liver and liver enzymes in patients with NASH [12]. However, fatty liver improvement by colesevelam treatment is not consistently recognized as an outcome because it has not been demonstrated in other reports [13]. In addition to LDL-C reduction, colesevelam can treat BA diarrhea [14], and a combination therapy of IBATi and BAS may prevent diarrhea as an IBATi side effect. Our study aimed to investigate EXB efficacy and safety in combination with CTM for patients with NAFLD.

2. Materials and methods

2.1. Trial design

The SPIRIT (Standard Protocol Items for Randomized Trials) statement and its checklist were followed in preparing the protocol. This trial is designed as single-center, randomized, double-blind, placebo-controlled, parallel-group, investigator-initiated study to investigate the efficacy and safety of a combination therapy of a 10 mg EXB tablet and 9 g CTM powder formulation (4 g CTM), an EXB monotherapy (10 mg), or a CTM monotherapy CTM (9 g), compared to that of placebo treatments. All treatments were administered orally once daily for 16 weeks in NAFLD patients. The experimental groups are as follows (Fig. 1): the EXB+CTM group (10 mg EXB and 9 g CTM), the EXB group (10 mg EXB and CTM placebo), the CTM group (EXB placebo and 9 g CTM), and the PBO group (EXB placebo and CTM placebo). Magnetic resonance imaging (MRI) will be performed at baseline and 16 weeks after intervention and evaluated by a blinded independent liver specialist (KI). The study plan includes recruiting 100 adult NAFLD patients from the Yokohama City University Hospital cohort.

2.2. Ethical considerations and registration

This study will be conducted in compliance with the Declaration of Helsinki, "Order for Enforcement of the Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices" and with Good Clinical Practice (GCP) standards. The study protocol and relevant supporting data were approved on November 26, 2019, by the institutional ethics committee prior to participant enrollment. The trial results will be reported according to the Consolidated Standards of Reporting Trials 2010 guidelines. This trial has been registered in the ClinicalTrials.gov registry (NCT04235205) and will be overseen by the external monitor and clinical research organization. Written informed consent for study participation will be obtained from all enrolled participants.

2.3. Study endpoints and rationale

The primary endpoint will compare absolute changes in LDL-C levels from baseline to 16 weeks after treatment initiation among patients receiving EXB-CTM combination therapy, EXB monotherapy, CTM monotherapy, or placebo (Table 1). Recent studies reported that serum and hepatic BA levels increase in NASH patients and that the severity of NASH is related to a specific change in the enterohepatic BA circulation [6]. Since the inhibition of BA reabsorption from the intestine is suggested to possibly improve NASH, we will focus on LDL-C reduction associated with increased BA production. LDL-C is commonly used for assessing NAFLD. For instance, phase II studies of VK2809 and obeticholic acid used LDL-C as primary endpoint [15], and a phase IIa study investigating EXB monotherapy for NAFLD in the U.S. used LDL-C as the primary endpoint. The NAFLD guidelines recommend using anti-dyslipidemic drugs to treat NAFLD. Because reducing LDL-C may improve cholesterol accumulation in the liver and is expected to improve NAFLD, LDL-C was considered an appropriate endpoint for monitoring NAFLD. Since CTM, which is proposed to be used in the current study, is a hypercholesterolemia treatment, and EXB is shown to reduce LDL-C in patients with chronic constipation, LDL-C is expected to be a sensitive surrogate for monitoring the efficacy of the EXB-CTM combination therapy.

Since this phase IIa study aimed to confirm the proof-of-concept (POC), it is appropriate to monitor treatment efficacy using LDL-C as surrogate marker that can be tested within a short time period rather than the histopathological assessment using a more invasive liver biopsy. The secondary endpoints include liver function and liver fat fraction assessments that will provide a comprehensive evaluation. After confirming the POC in this study, we plan to perform drug-dosing studies during the next phase, including liver biopsy analysis for determining the NASH status and hepatic fibrosis after long-term drug administration.

Our current secondary endpoint will determine the absolute change from baseline to 16 weeks after treatment initiation in liver fat fraction and hepatic fibrosis measured by MRI-PDFF and

magnetic resonance elastography (MRE) according to our previous method [16, 17], respectively. Monitoring of other parameters as secondary endpoints for absolute changes between baseline and 16 weeks after treatment initiation will include alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (γ-GTP), high-density lipoprotein cholesterol (HDL-C), non-HDL-C, LDL-C/HDL-C ratio, and triglyceride. Other monitored variables are adverse events (AEs), the results of standard laboratory analysis, physical examination, vital signs, and compliance rate. Physical assessments will be performed and evaluated at Yokohama City University, using standard procedures.

Table 1. Study endpoints

Primary endpoint	**Secondary endpoints**	**Exploratory endpoints**
<Efficacy endpoint>	**<Efficacy endpoint>**	**<Efficacy endpoint>**
・Absolute change in serum LDL-C from baseline to Week 16	・Absolute change in the liver fat fraction (%), as measured by MRI-PDFF, from baseline to Week 16	<Class; lipid> Change in the following from baseline to Week 16: serum lipid fraction[a], apoprotein A1 or B.
	・Absolute change in hepatic fibrosis, as measured by MRE, from baseline to Week 16	<Class; Endocrine> Change in the following from baseline to Week 16: serum c-peptide, plasma total GLP-1, plasma active GLP-1 and plasma adiponectin.
	・Change in the following from baseline to Week 16: ALT, AST, γ-GTP, HDL-C, non-HDL-C, LDL-C/HDL-C ratio and TG	<Class; Bile acid> Change in the following from baseline to Week 16: serum C4, FGF-19, total bile acid and fraction of bile acid and fecal bile acid (total and fraction)
		<Class; Inflammation> Change in the following from baseline to Week 16: plasma

		CK18 fragment M30, endotoxin activity assay, plasma LBP.
	<Safety endpoint>	<Class; fibrosis> Change in the following from baseline to Week 16: serum type IV collagen 7s, PIIIP, hyaluronic acid and TIMP-1
	・Incidence of adverse events	<Class; gut-microbiota> Change in fecal gut-microbiota from baseline to Week 16
	・Change in the daily score of the BSFS, number of bowel movements, and PAC-QOL from baseline at every visit	<Class; other> Change in serum TMAO from baseline to Week 16
	・Change in the CLDQ scale from baseline at every visit	

All objectives will be compared among combination therapy of EXB (10 mg) and CTM (9 g), monotherapy of EXB (10 mg) or CTM (9 g) and placebo groups. ALT, alanine transaminase; AST, aspartate transaminase; BSFS, Bristol Stool Form Scale; CLDQ, Chronic Liver Disease Questionnaire; CTM, cholestyramine; EXB, elobixibat; FGF-19, fibroblast growth factor 19; γ-GTP, γ-glutamyl transpeptidase; GLP-1, glucagon-like peptide-1; HDL-C, high-density lipoprotein cholesterol; LBP, lipopolysaccharide-binding protein; LDL-C, low-density lipoprotein cholesterol; PAC-QOL, Patient Assessment of Constipation Quality of Life; PIIIP, procollagen-3-peptide; TIMP-1, tissue inhibitor of metalloproteinase-1; TMAO, trimethylamine N-oxide.

[a] chylomicron-cholesterol, chylomicron-TG, intermediate density lipoprotein cholesterol, very low-density lipoprotein cholesterol (VLDL-C), LDL-C, HDL-C, LDL-TG, VLDL-TG.

2.4. Rationale for treatment dose, mode, and duration

It was considered appropriate to use the currently approved dose of each drug in our study to investigate the effects of the EXB-CTM combination therapy in an exploratory manner and provide

an adequate and safe experience under clinical management. Although it has not been previously published in the literature or presented at academic meetings, it is becoming known widely in clinical settings in Japan that treating NAFLD patients with this combination therapy at the approved dosing provides improvement of hepatic parameters and follow-up without adverse drug reactions, including diarrhea and constipation. The approved dosing should be sufficient to ensure safety and tolerability in NAFLD patients and allow an efficacy evaluation of the combination therapy.

Although the hypercholesterolemia regimen is 9 g/dose, two to three times daily, the dose in this study was taken once daily before breakfast concomitant with EXB. This regimen was based on the consideration that combination therapy is preferable for achieving continuous drug compliance, along with reducing AEs due to EXB, including abdominal pain and diarrhea.

Global exploratory NAFLD trials have adopted a treatment period of at least 12 weeks (Safety, Tolerability, Pharmacokinetics and Efficacy of LMB763 in Patients With NASH: NCT02913105; A Study of the Efficacy and Safety of CF102 in the Treatment of Non-Alcoholic Fatty Liver Disease: NCT02927314). A Japanese phase III study of EXB in patients with chronic constipation demonstrated that LDL-C levels rapidly decreased after two treatment weeks, and lower levels were maintained for 52 weeks at least [7]. However, this study plans using MRI examination as a secondary endpoint, which requires a treatment period minimum for efficacy determinations. Thus, a 16-week treatment period was incorporated into the study design to ensure appropriate EXB efficacy evaluation.

2.5. Drug supply

Only the Patient Enrolment Centre will know the treatment allocation, and double-blinding of the physicians and patients will be maintained throughout the study until all patients have completed the 16-week study, and the database has been locked for all study data. Tablets of EXB (5 mg) and the corresponding reference placebo, which is indistinguishable in appearance, were manufactured and supplied by EA Pharma Co., Ltd (Tokyo, Japan). The CTM powder, 9 g/dose (CTM 4 g), was purchased from the market, and its reference placebo, which is indistinguishable in appearance, was manufactured and both were supplied by TOYO Pharmaceutical Co., Ltd. (Osaka, Japan). For the study drug prescriptions, the physicians will enter the drug allocation number provided by the Patient Enrolment Centre on the prescription form. The drug manager will dispense the study drug to the patient with the drug allocation number. Exceptions to blinding will be to secure a patient's safety and for treatment upon request to the Patient Enrolment Centre personnel by the principal investigator.

2.6. Sample size estimation

Due to the exploratory nature of this study, no formal power calculations were used to determine

sample sizes. The number of subjects (100 patients in total, 25 patients per treatment group) was chosen based on data from Japanese phase III clinical study and a long-term study on EXB in patients with chronic constipation [7]. The phase III study showed an LDL-C level reduction after 2 treatment weeks, while the long-term study demonstrated that similar reductions persisted from week 4 to week 52 of treatment. Hence, we hypothesize that the reduction in LDL-C associated with EXB treatment (10 mg) will remain the same between week 2 and 16. As the mean difference in LDL-C level change from baseline of between the 10 mg EXB group and placebo group was 12.4 mg/dL, the pooled standard deviation was predicted as 15.5 mg/dL. The LDL-C level change from baseline to week 2 in the 10 mg EXB group and the placebo group was 16.6 mg/dL and 4.2 mg/dL, respectively. Using sample sizes of n=20 per group, the study has 80% power with one-sided α=0.05. At an expected dropout rate of 10%, 23 patients per treatment group will be randomized in this study, which should support an assessment of the EXB-CTM combination therapy effects.

2.7. Eligibility

The physicians will enter the patients into the Screening List, assign an identification code to each patient, and determine eligibility according to the inclusion and exclusion criteria (Table 2). If no eligibility issues are identified, the investigator or sub-investigator and investigative staff will enter the necessary information into the Electronic Data Capture (EDC) system for enrolment. The patient enrolment number will then be assigned, and enrolment will be completed.

Table 2. Patient Inclusion and Exclusion Criteria

	Inclusion Criteria	**Exclusion Criteria**
1	Patients who received adequate explanation about this study and provided written informed consent	Women who are pregnant, breastfeeding, possibly pregnant or do not agree to use birth control during the study
2	Patients who are ≥ 20 and < 75 years of age at the time of informed consent	BMI <23 kg/m2

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3	Patients who have a current biopsy-confirmed NASH within 8 months of screening or a suspected diagnosis of NAFLD/NASH based on the criteria outlined below: (1) Biopsy-confirmed NASH is defined as histological NASH diagnosis with fibrosis stage F1 through F3 and a NAFLD activity score (NAS) of ≥4 with a score of ≥1 in each of the NAS components below as assessed by a pathologist using the NASH Clinical Research Network criteria: 1) Steatosis (scored 0 to 3) 2) Ballooning degeneration (scored 0 to 2) 3) Lobular inflammation (scored 0 to 3) (2) The suspected diagnosis of NAFLD/NASH is based on the following criteria: 1) AST ≥20 U/L and ALT ≥40 U/L in males or ≥28 U/L in females 2) Waist circumference ≥85 cm in males or ≥90 cm in females 3) Diagnosis of metabolic syndrome having 2 or more of the following 3 risk factors at Screening: a): Fasting plasma glucose ≥110 mg/dL or undergoing drug treatment for elevated glucose b): SBP ≥130 mmHg and/or DBP ≥85 mmHg or undergoing drug treatment for hypertension, or antihypertensive drug treatment in a patient with a history of hypertension c): TGs ≥150 mg/dL or	Liver stiffness measured by MRE >6.7 kPa

	undergoing drug treatment for elevated TGss, and/or HDL-C <40 mg/dL or undergoing drug treatment for reduced HDL-C	
4	Screening MRI -PDFF with ≥8% liver steatosis	Any of the following laboratory abnormalities: (1) ALT >5 × ULN or AST >5 × ULN (2) PT-INR ≥1.3 unless on anticoagulant therapy (3) Total bilirubin > ULN, except with an established diagnosis of Gilbert's syndrome (4) Platelet count < 80,000/μL (5) eGFR <45 as calculated by the BSA adjustment (normalized eGFR)

5	Fasting serum LDL-C >120 mg/dL or undergoing antidyslipidemic drugs	Acute or chronic liver disease other than NAFLD/NASH including but not limited to the following: (1) Hepatitis B (as defined by the presence of HBs antigen at Screening) or hepatitis C (as defined by the presence of HCV antibody [anti-HCV]) Patients with positive anti-HCV who test negative for HCV ribonucleic acid (HCV-RNA) at Screening will be allowed to participate in the study as long as there is evidence of viral negativity for a minimum of 12 months prior to Screening (2) Evidence of AIH (3) History of PBC, PSC, Wilson's disease, alpha-1-anti-trypsin deficiency, hemochromatosis or iron overload, drug-induced or ALD, or known bile duct obstruction (4) Suspected or proven HCC
6	Be willing to maintain a stable diet and physical activity throughout the course of the study	Known history of HIV
7		Medical history of liver cirrhosis
8		Clinical evidence of portal hypertension to include any history of ascites, hepatic encephalopathy, or presence of esophageal varices
9		Use of drugs historically associated with NAFLD (amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, or valproic acid) or other known hepatotoxins for ≥2 weeks in the year prior to Screening

10		Use of the following medications: (1) GLP-1 agonists unless on a stable dose 3 months prior to Screening or liver biopsy (2) Ursodeoxycholic acid or thiazolidinediones within 3 months prior to Screening (3) Antidyslipidemic drugs have been stable for ≥3 months prior to Screening (4) Oral antidiabetic drugs have been stable for ≥3 months prior to Screening (5) Agents (including herbal over-the-counter weight loss preparations) or medications known to significantly impact body weight within 3 months prior to Screening
11		History of significant alcohol consumption, defined as an average of ≥20 g/day in female patients and ≥30 g/day in male patients, for a period of >3 consecutive months within 1 year prior to Screening, hazardous alcohol use (Alcohol Use Disorders Identification Test score ≥8), or an inability to reliably quantify alcohol consumption but determined as alcohol polydipsia based upon judgment of the Investigator or subinvestigator
12		Weight change ≥10% within the 6 months prior to Screening or ≥5% within the 3 months prior to Screening

13		Surgery planned during the study period or after bariatric surgery (e.g., gastroplasty and Roux-en-Y gastric bypass)
14		Type 1 diabetes by medical history
15		Uncontrolled Type 2 diabetes defined as hemoglobin A1c (HbA1c) >9.5% at Screening (patients with HbA1c >9.5% may be rescreened) or requiring insulin dose adjustment >10% within 2 months prior to Screening
16		Clinical hyperthyroidism or hypothyroidism or Screening hormone results pointing to thyroid dysfunction. Patients receiving dose-stable thyroid replacement therapy for ≥3 months prior to Screening will be allowed to participate in this study as long as thyroid tests show that the patient is euthyroid and stable
17		History of any condition causing malabsorption such as chronic pancreatitis, extensive bowel/small intestine surgery, celiac disease, or bile flow obstruction
18		History of any condition associated with acute or chronic diarrhea such as IBD, functional diarrhea, IBS with predominant diarrhea, IBS with mixed bowel habits, or unclassified IBS
19		Uncontrolled hypertension (either treated or untreated) defined as SBP >160 mmHg or a DBP >100 mmHg at Screening
20		History of New York Heart Association (NYHA) Class III or IV heart failure, or known left ventricular ejection fraction <30%

21		History of myocardial infarction, unstable angina, percutaneous coronary intervention, coronary artery bypass graft, or stroke or major surgery within 6 months prior to Screening
22		Active substance abuse, within 1 year prior to Screening
23		Participation in an investigational new drug trial in the 30 days prior to Screening or within 5 half-lives of an investigational agent, whichever is longer
24		Complication with malignancy Patients with a history of malignancies that have been treated with curative intent or completed chemotherapy may be eligible. Patients under evaluation for malignancy are not eligible
25		Known intolerance to MRI or conditions contraindicated for MRI procedures
26		Any other condition which is considered to be inappropriate for the study by the Investigator or subinvestigator

AIH, autoimmune hepatitis; ALD, alcoholic liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BSA, body surface area; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide-1; HBs, hepatitis B surface; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDL-C, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; LDL-C, low-density lipoprotein cholesterol; MRE, magnetic resonance elastography; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; PDFF, proton density fat fraction; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; PT-INR, prothrombin time-international normalized ratio; SBP, systolic blood pressure. TG, triglyceride, ULN, upper limit normal

2.8. Randomization and masking

The patients will be randomized to each group (EXB+CTM, EXB, CTM, and PBO) at a ratio of

1:1:1:1 using a computer-generated centrally administered procedure. The applicable drug number will be assigned via the EDC. All trial drugs will be packaged identically and identified only by a number. As noted above, the treatment assignments will be fully masked from the patients and physicians.

2.9. Keycode break

If the investigator or sub-investigator considers it urgently necessary to break the study keycode prematurely, they will contact the person responsible for study drug randomization to file the request. This may occur due to a serious AE (SAE), the need for treating an AE, or a similar situation.

2.10. Harms and AE monitoring

AEs are defined as any unfavorable or unintended sign (including laboratory parameters and abnormal vital signs), symptom, or disease that may occur during the study period. AEs may develop that are not directly related to the study drugs. The investigator or sub-investigator will assess the severity of the AEs. Any AE that meets any of the following criteria will be considered an SAE: death, life-threatening, requirement of hospitalization or prolonged hospitalization for treatment, disability, threat of disability, other serious conditions, congenital disease, or anomaly in offspring. If an SAE occurs, the investigator or sub-investigator will appropriately treat it, and the investigator will immediately report the details to the Hospital Director and the study drug supplier.

2.11. Study procedures

The investigator or sub-investigator will perform all observations, tests, investigations, and evaluations according to the descriptions provided in Table 3. If a blood test is scheduled for a study visit, blood collection will only be performed after the patient had fasted for at least eight hours.

Table 3. Schedule of observations, tests and assessments

	Before Screening	Screening	Treatment Period					Follow-up
		V1	V2/Randomization	V3	V4	V5	V6/EOT	V7
Study Week		Week -8 to Day -1	Day -1	Week 4	Week 8	Week 12	Week 16	EOT to 2 weeks
Visit Window			-	±7 days	±7 days	±7 days	±7 days	±7 days

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Informed consent	○							
Inclusion/exclusion criteria		○	○					
Demographics		○						
Serology [a]		○						
Chest X-ray		○						
ECG		○						
Physical examination [b]		○	○				○	
Vital signs [c]		○	○	○	○	○	○	○
Pregnancy test [d]		○	○				○	
MRI [e]		○					○	
Liver biopsy		△						
Randomization			○					
Hematology/urinalysis [f]		○	○[k]	○	○	○	○	○
Endocrinology		○						
Biochemistry 2 [g]		○	○[k]				○	
Total bile acids			○[k]				○	
Lipid profile		○	○[k]	○	○	○	○	○
Others [h]			○[k]				○	
Blood/stool sampling for storage			●				●	
Dispense study drug			○	○	○	○		
Review study drug compliance				○	○	○	○	
Review alcohol consumption [i]		○						
Review concomitant medications		○	○	○	○	○	○	

Review adverse events		○	○	○	○	○	○	○
Questionnaire/Review patient diary[j]			○	○	○	○	○	

○: To be performed

△: Information will be collected from the patients who have liver biopsy results.

●: Blood/stool sampling for storage will be collected for analysis of genes, fibrosis, inflammation, etc. upon separate consent.

[a]: Includes HBs antigen, HCV antibody and HCV-RNA.

[b]: Includes height (at V1 only), body weight, waist circumference, and waist-to-hip ratio. BMI will be calculated based on height and weight.

[c]: Vital signs include blood pressure, heart rate, respiratory rate, and axillary temperature.

[d]: For women of childbearing potential, a urinary pregnancy test will be performed at V1, V2 and V6. The test is not required at V2 when it occurs within 1 month after V1.

[e]: Patients will have MRI to measure liver fat (PDFF), and total liver volume. Patients who discontinue before V6 (Week 16) should have an MRI performed at End of Treatment if they completed at least 4 weeks of treatment.

[f]: Hematology/urinalysis includes hematology/coagulation, biochemistry 1 and urinalysis.

[g]: Biochemistry 2 includes glucose, HbA1c and insulin.

[h]: Others include high-sensitivity CRP, Type IV collagen 7s, FIB-4 and APRI.

[i]: History of alcohol consumption will be obtained at Screening.

[j]: Questionnaires of PA-QOL and CLDQ will be performed at each visit. Number of bowel movements, BS score, etc. will be collected from the patient diary.

[k]: Data within 1 month can be used as substitute.

2.12. Criteria and procedure for withdrawal from the study

The investigator or sub-investigator will discontinue the study enrollment of a patient if the participant meets any of the following criteria: (1) The patient desires withdrawal. (2) The patient is found after enrolment not to meet the inclusion criteria or to meet the exclusion criteria. (3) It is the opinion of the investigator or sub-investigator that having the patient continue in the study is not appropriate due to an AE. (4) It is the opinion of the investigator or sub-investigator that having the patient continue in the study is not appropriate due to any other reason.

2.13. Efficacy evaluation

The primary efficacy endpoint will be the absolute change in LDL-C levels from baseline to 16 weeks after treatment initiation. The secondary and other efficacy endpoints are provided in Table 1.

The assessment of the MRI-PDFF/MRE will be performed by an independent liver specialist that is blinded to the treatment (IK).

2.14. Safety assessments

The following safety evaluations will be performed during each patient visit from the time of drug treatment initiation until the 2-week follow-up period: (1) Incidence of AEs in the treatment groups compared with that of the placebo groups. (2) Change in the daily score of the Bristol Stool Form Scale from baseline regarding the number of bowel movements and the Patient Assessment of Constipation Quality of Life (PAC-QOL). (3) Change from baseline of the Chronic Liver Disease Questionnaire (CLDQ) scale.

2.15. Populations analysis

The set of subjects to be analyzed will be determined prior to the data lock of each patient and will be defined as follows. The full analysis set (FAS) will be used for assessment of the primary efficacy analysis. The FAS will include all patients who are randomized, except those who meet any of the following criteria: (1) Patients with major protocol deviation (e.g., deviation of informed consent, major deviation in the study procedures), (2) Patients who have not received any dose of study drug, (3) Patients who have no measurement for the efficacy endpoint. The Safety Analysis Set (SAS) will be used for major analysis for safety assessment and will include all patients who receive at least one dose of study drug.

2.16. Statistics analysis

Considering the placebo-controlled randomized parallel-group comparison design, we will use descriptive statistics to summarize differences between the placebo group and each monotherapy or combination group and those between each monotherapy group and the combination group. Unless otherwise noted, the descriptive statistics include the number of patients, mean, standard deviation, median, minimum and maximum values for continuous variables, frequency, and percentage for categorical variables.

For the primary endpoint, the change in serum LDL-C from baseline to Week 16 (difference of measurement values between time points) will be summarized for each treatment group. Point estimates and confidence intervals for the mean difference of the change from baseline between the placebo group and each monotherapy or combination group and those between each monotherapy group and the combination group will be calculated. The changes over time in measured parameters will be summarized for each treatment group. The ratio of change (ratio of the absolute change to the baseline value) will also be analyzed using the same methods. For secondary endpoints, the analysis will be performed similar to the methods used for the primary endpoint. Due to the exploratory

nature of this study, a concrete statistical hypothesis is not formulated. For exploratory purposes, in case any statistical test is performed or confidence interval of any parameter is calculated, the significance level of 5% (two-sided) and the confidence coefficient of 95% will be used. Multiplicity arising from the interpretation of statistical tests or confidence intervals will not be adjusted.

2.17. Interim analysis

Not applicable.

2.18 Data management, central monitoring and audit

The investigators sites will maintain individual records for each patient as source data, which include a copy of informed consent, medical records, laboratory data and other records or notes. All data will be collected by the independent data management center. The data management center will oversee the interstudy data sharing process. The clinical data entry, data management and central monitoring will be performed using the electric data capture VIEDOC 4 (PCG Solutions, Sweden). Also, auditing will be planned at external clinical research organization in this study.

2.19. Efficacy and Safety Assessment Board

An Efficacy and Safety Assessment Board will be established to ensure the overall monitoring and safety review of this study. The Board will consist of three external board members who have no relationship with the operations involved in the study.

2.20. Study flow and schedule of enrolment, interventions, and assessments

A study flowchart is shown in Fig. 1. The study schedule is presented in Table 3.

3. Discussion

This is the first proof-of-concept study proposed to explore the effect of EXB in combination with CTM in patients with NAFLD. A previous study showed that an IBATi improved both hepatic and whole-body aspects in a mouse model of NAFLD [9]. However, EXB causes AEs, including abdominal pain and diarrhea, by increasing colonic BAs, resulting in the development of BA-induced diarrhea. Therefore, monotherapy with EXB may decrease in safety and tolerability for NAFLD patients. Considering the MOA, the removal of increasing BAs from the colon using CTM has the potential to improve BA-induced diarrhea.

In some well-known trials (e.g., PIVENS, FLINT, and GOLDEN) [15, 18-20], the primary endpoints included liver histology, which was evaluated using liver biopsy specimens. Liver histology

endpoints, such as the complete resolution of NASH, are considered surrogates for preventing cirrhosis because they are considered predictive of clinical benefit but are not direct measures. Presently, as described in the guidelines ("the gold standard"), the only way to diagnose NASH is via a liver biopsy. However, performing liver biopsies in all patients with NAFLD is impossible as there are more than 10 million patients with NAFLD in Japan. In addition, there are various problems associated with liver biopsies, such as invasiveness, cost, and misinterpretation between pathologists.

Recently, significant advances have been made regarding MRI techniques, and hepatic steatosis and fibrosis can be diagnosed at extremely high sensitivity and specificity using MRE [21, 22]. It is also possible to quantify the fat ratio using MRI by measuring the PDFF value using the iterative decomposition of water and fat with echo asymmetry and least-squares estimation sequence (IDEAL IQ) method [17, 23]. MRE and MRI-PDFF can be performed simultaneously in one imaging session, and by combining the results, hepatic steatosis and hepatic fibrosis can be evaluated. MRI-based noninvasive assessment of liver fibrosis and steatosis would be a potential alternative to liver biopsy in clinical practice [17]. Hence, we chose MRI for the noninvasive assessment of hepatic steatosis in this study. Quantification of liver fat content via MRI-PDFF has been previously shown to be sensitive in detecting changes and has been used in nonalcoholic steatohepatitis clinical trials for quantitative fat assessment [17, 21]. Using paired MRI-PDFF and liver histology data, Patel and colleagues have shown that an absolute change in liver fat content of −4.1% with a relative change of −29.3% is associated with histological improvements of a 2 or more point reduction in NAFLD Activity Score (NAS), with 1 point each for steatosis and ballooning [16]. This suggests that changes in liver fat may also be a surrogate for early hepatocellular injury. Furthermore, weight-loss studies have demonstrated a clinically meaningful decrease of 4.7–4.8% in absolute liver fat content between the beginning and end of the intervention [24]. Based on these considerations, and consistent with recent trials in the field, we chose ≥5% reduction in absolute liver fat content as responder criteria for primary outcome and a relative 30% reduction as a clinically meaningful change in liver fat content.

Our study has several strengths: (1) the first randomized, placebo-controlled, double-blinded study focused on removing BAs in patients with NAFLD; (2) a novel POC study considering the drug MOA for improving the treatment effect and tolerability, as well as reducing the AEs; (3) MR images are captured following a standardized protocol and processed under the supervision of a hepato-radiologists blinded to the study; (4) the comparison of colocalized regions of interest for fat changes in each of the nine liver segments between week 0 and week 16; (5) the final assessments of the MRI examinations were randomized with regards to time points in order to reduce bias; and (6) the measurement of exploratory endpoints such as the lipid class, endocrine functions, BA, inflammation, fibrosis, gut-microbiota, and trimethylamine-N-oxide (Table 2). Nevertheless, our study also has limitations as follows: (1) performed within a single center; (2) a relatively small sample size; (3) a

relatively short treatment duration; and (4) the lack of liver biopsy.

NAFLD/NASH is a heterogeneous disease with correspondingly complex pathophysiology, which includes redundant pathways that may not be uniform across patients. Considering the MOA of pharmaceuticals for treating NAFLD/NASH, its complex pathophysiology permits the development of a wide array of potentially viable therapeutic targets, especially components of anti-metabolic, anti-inflammatory, and anti-fibrotic pathways [25]. In a phase IIa trial for NASH/NAFLD therapies, BMS-986036 (FGF21) [26] and NGM282 (FGF19) [27] exerted an anti-metabolic effect, and the liver fat content was used as the primary endpoint. Although current NAFLD therapy focuses on anti-metabolic, anti-inflammatory, and anti-fibrotic aspects in the liver as MOA, diminishing BAs for treating NAFLD is a novel therapeutic target for patients with NAFLD. EXB-CTM combination therapy-associated improvements in the changes of the absolute and relative liver fat levels changes appeared to be clinically relevant.

4. Conclusion

This will be the first POC study to assess the efficacy and safety of the EXB-CTM combination therapy in patients with NAFLD. We hypothesize that the EXB-CTM combination therapy will have higher efficacy and fewer side effects than either monotherapy.

Declarations

Ethics approval and consent to participate

The Ethics Committee of Yokohama City University Hospital approved the study protocol on November 26, 2019. This trial is registered with ClinicalTrials.gov (number NCT04235205). Informed consent will be obtained from all participants prior to enrolment. Any information supplied to gain informed consent was also approved by the Ethics Committee.

Confidentially

Data will be retained in accordance with the Japanese ethical guidelines for clinical research. Participants will be allocated a unique identification (ID) number at entry. The master list linking participant personal information and ID number will be maintained in a separate locked cabinet and password-protected hard drive. Data will be analyzed by ID number only. Records will be retained for 5 years after study completion and then destroyed by the data center.

Consent for publication and dissemination

The results of this study will be submitted for publication in international peer-reviewed journals and the key findings presented at conferences. Participants will be informed of the results of the trial by the investigators. Authorship will be ascribed in accordance with the International Committee of Medical Journal Editors guidance.

Competing interests

AN report grants and research support from Gilead, Mylan EPD, EA Pharma, Kowa, Taisho, Biofermin; is a consulting adviser for Gilead, Boehringer Ingelheim, BMS, Kowa, Astellas, EA Pharma, Mylan EPD. Other authors declare no competing interests.

Funding

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Authors' contributions

TKessoku and AN participated in study design. TKessoku and TKobayashi conducted feasibility phase work. Recruitment of participants and follow-up will be performed by TKessoku, AO, MI, TKobayashi, YH, YO, MY, and SS. Reading of MRI will be done by KI. Exploratory item will be measured by HU and KW. TKessoku, TKobayashi, KY, TY, and AN will participate in data interpretation. All authors contributed to writing, and all read and approved the final manuscript.

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Data sharing statement

Researchers can apply for data by submitting a proposal to the corresponding author.

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Figure legend

Figure 1. Study design. [a]N = 100 enrolled. CTM, cholestyramine; EXB, elobixibat; NAFLD, nonalcoholic fatty liver disease; PBO, placebo; QD, quaque die.

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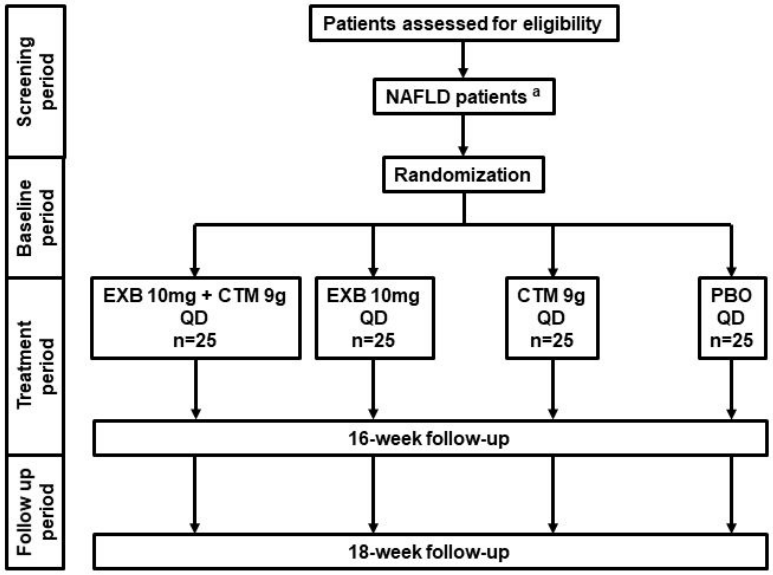

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Figure 1

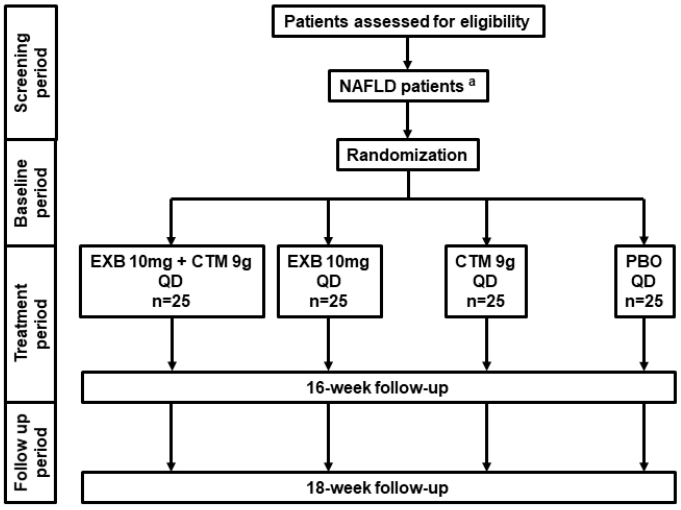

Figure 1

254x190mm (96 x 96 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2,5,6
	2b	All items from the World Health Organization Trial Registration Data Set	5,6
Protocol version	3	Date and version identifier	2
Funding	4	Sources and types of financial, material, and other support	23
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,23
	5b	Name and contact information for the trial sponsor	23
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	23
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	6,23
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4,5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10-16
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	21
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	19
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	17,18
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10-16
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6-8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	17-19, Fig.1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9,10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9,10
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	16,17
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	16,17

Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	16,17
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	16,17
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	16,17
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	17-19
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	17-19
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	19-21
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	20-21
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	20-21
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	20-21
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	17
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	21
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17

Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	17-18
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	5,6,23
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	23
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	23
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	23
	31b	Authorship eligibility guidelines and any intended use of professional writers	23
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Rationale and design of a randomized, double-blind, placebo-controlled, parallel-group, investigator-initiated phase 2a study to investigate the efficacy and safety of elobixibat in combination with cholestyramine for nonalcoholic fatty liver disease

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Manuscript ID	bmjopen-2020-037961.R1
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Kessoku T et al.

Rationale and design of a randomized, double-blind, placebo-controlled, parallel-group, investigator-initiated phase 2a study to investigate the efficacy and safety of elobixibat in combination with cholestyramine for nonalcoholic fatty liver disease

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Study dates: November 1, 2019 to December 31, 2020.

ABSTRACT

Introduction: Nonalcoholic fatty liver disease (NAFLD) pathogenesis involves abnormal metabolism of cholesterol and hepatic accumulation of toxic free-cholesterol. Elobixibat (EXB) inhibits the ileal bile acid (BA) transporter. EXB and cholestyramine (CTM) facilitate the removal of free cholesterol from the liver by decreasing BA recirculation to the liver, thereby stimulating novel BA synthesis from cholesterol. In this randomized, double-blind, placebo-controlled, parallel-group, phase IIa study, we aim to provide a proof-of-concept assessment by evaluating the efficacy and safety of EXB in combination with CTM in patients with NAFLD.

Methods and analysis: A total of 100 adult patients with NAFLD, diagnosed based on low-density lipoprotein cholesterol (LDL-C) level of >120 mg/dL and liver fat content of ≥8% by magnetic resonance imaging (MRI)-based proton density fat fraction (MRI-PDFF), who meet the inclusion/exclusion criteria will be enrolled. The patients will be randomly assigned to receive the combination therapy of 10 mg EXB and 9 g CTM powder (4 g CTM), 10 mg EXB monotherapy, 9 g CTM powder monotherapy, or a placebo treatment (n = 25 per group). Blood tests and MRIs will be performed 16 weeks following treatment initiation. The primary study endpoint will be the absolute LDL-C level change at week 16 after treatment initiation. The secondary endpoint will include absolute changes in the liver fat fraction as measured by MRI-PDFF. This proof-of-concept study will determine whether the combination therapy of EXB and CTM is effective and safe for patients with NAFLD.

Ethics and dissemination: Ethics approval was obtained from the Ethics Committee of Yokohama City University Hospital before participant enrollment. The results of this study will be submitted for publication in international peer-reviewed journals and the key findings will be presented at international scientific conferences.

Trial registration: This trial is registered with ClinicalTrials.gov (number NCT04235205).

Protocol version: 1.3, February 20, 2020

Keywords: Nonalcoholic fatty liver disease, elobixibat, cholestyramine, combination therapy, low-density lipoprotein cholesterol, phase 2a

Strengths and limitations of this study

- This is the first proof-of-concept study to assess the efficacy and safety of the combination therapy of elobixibat and cholestyramine in patients with nonalcoholic fatty liver disease.
- This is a randomized, double-blind, placebo-controlled, parallel-group, phase IIa study.
- The primary study endpoint is the change in the absolute low-density lipoprotein cholesterol level.

- The secondary endpoint is the absolute changes in the liver fat fraction measured by magnetic resonance imaging proton density fat fraction.
- Our study has the following limitations: (1) performed in a single center; (2) a relatively small sample size; (3) a relatively short treatment duration; and (4) the lack of liver biopsy.

1. INTRODUCTION[1]

Nonalcoholic fatty liver disease (NAFLD) is a clinical condition that is detected by tissue or image analyses and diagnosed by excluding alcoholism and other liver diseases. It is the hepatic manifestation of a metabolic syndrome and is often associated with obesity, diabetes mellitus, dyslipidemia, hypertension, and other disorders. The prevalence of NAFLD is increasing worldwide; in Japan, it increased from 12.9% in 1994 to approximately 34.7% in 2000.[1] NAFLD is classified as nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH), which includes inflammation and a progressive disease associated with liver cancer or cirrhosis, affecting 10%–20% of the patients.[2] The factors contributing to NASH pathogenesis are abnormal cholesterol metabolism and free cholesterol accumulation in the liver, which is toxic to hepatocytes, leads to inflammation and fibrosis.[3]

Bile acids (BAs) may be critical in NASH pathogenesis.[4-6] They act as signaling molecules in lipid, glucose, and energy homeostasis, whose metabolic pathways are linked to NAFLD/NASH and comorbidities, including metabolic syndrome, obesity, and diabetes. The BAs and free cholesterol, as the precursor for BA synthesis, can act as lipotoxic agents that drive inflammation and fibrosis. In previous studies, both serum and liver BA levels were elevated in patients with NASH, and recent data suggested that the occurrence and severity of NASH are associated with specific changes in circulating BAs.[6] Therefore, targeting the BA pathways may have a therapeutic potential for patients with NAFLD.

Elobixibat (EXB) inhibits the ileal BA transporter (IBAT) and is approved for marketing as an oral treatment for chronic constipation.[7] The IBAT, expressed primarily in the distal ileum, is a key element in the enterohepatic circulation of BAs, as it facilitates BA reabsorption. EXB is orally administered and acts locally in the gut, where it reversibly binds to IBAT to decrease the re-uptake of BAs to the liver. EXB at expected therapeutic doses has minimal systemic exposure. The pharmacodynamic assessment of EXB in a Japanese phase I study showed decreased serum LDL-C levels in groups that received repeated doses of ≥5 mg EXB.[8] In addition, a study on NAFLD mouse model reported that treatment with another IBAT inhibitor (IBATi) promoted BA

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[1]**Abbreviations**

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FAS, full analysis set; γ-GTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MOA, mechanism of action; MRI-PDFF, magnetic resonance imaging-based proton density fat fraction; MRE, magnetic resonance elastography; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; POC, proof-of-concept; SAS, safety analysis set.

biosynthesis from cholesterol, which accumulated in the liver of mice that received a high-fat diet.[9] Furthermore, converting the BA pool in the liver into hydrophobic BAs decreases hepatic cholesterol accumulation via farnesoid X receptor (FXR) activation, thereby improves the NAFLD status.[9] EXB is also expected to improve the NAFLD status as it has the same mechanism of action (MOA) and lowers the LDL-C levels. However, increased BA level in the large intestine may stimulate the secretion of water and electrolytes in the intestinal tract and movement of the gastrointestinal tract, which induces BA diarrhea and promotes peristalsis.[10, 11]

The BA sequestrant (BAS) cholestyramine (CTM) is a strong base anion exchange resin, and its drug product alleviates hypercholesteremia and eliminates active leflunomide metabolites from the body. CTM inhibits the absorption of exogenous cholesterol by binding to BA in the intestinal tract, which increases feces excretion. CTM also promotes cholesterol catabolism to BA in the liver to compensate for the decreased BA level due to increased excretion. This ultimately decreases the LDL-C level. These effects are expected to promote hepatic lipid metabolism. In a Japanese clinical study, colesevelam, another BAS, improved fatty liver and liver enzymes in patients with NASH.[12] However, the improvement in fatty liver with colesevelam treatment is not consistently recognized as an outcome because it has not been demonstrated in other studies.[13] In addition to LDL-C reduction, colesevelam can alleviate BA diarrhea,[14] and a combination therapy of IBATi and BAS may prevent diarrhea, an adverse effect of IBATi. Our study aims to investigate the efficacy and safety of EXB in combination with CTM in patients with NAFLD.

2. MATERIALS AND METHODS

2.1. Trial design

The Standard Protocol Items for Randomized Trials (SPIRIT) statement and its checklist were followed in preparing the study protocol. This trial is designed as a single-center, randomized, double-blind, placebo-controlled, parallel-group, investigator-initiated study to investigate the efficacy and safety of a combination therapy of 10 mg EXB tablet and 9 g CTM powder formulation (4 g CTM), an EXB monotherapy (10 mg), or a CTM monotherapy CTM (9 g), compared with those of placebo treatments. All treatments will be administered orally once daily for 16 weeks to patients with NAFLD. The experimental groups will be as follows (Fig. 1): the EXB+CTM group (10 mg EXB and 9 g CTM), the EXB group (10 mg EXB and CTM placebo), the CTM group (EXB placebo and 9 g CTM), and the PBO group (EXB placebo and CTM placebo). Magnetic resonance imaging (MRI) will be performed at the baseline and 16 weeks after intervention and the data will be evaluated by a blinded independent liver specialist (KI). The study plan involves recruiting 100 adult patients with NAFLD from the Yokohama City University Hospital cohort.

2.2. Ethical considerations and registration

This study will be conducted in compliance with the Declaration of Helsinki, "Order for Enforcement of the Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices," and Good Clinical Practice (GCP) standards. The study protocol and relevant supporting data were approved on November 26, 2019, by the institutional ethics committee before participant enrollment. The trial results will be reported according to the Consolidated Standards of Reporting Trials 2010 guidelines. This trial has been registered in the ClinicalTrials.gov registry (NCT04235205) and will be overseen by an external monitor and clinical research organization. Written informed consent for study participation will be obtained from all enrolled participants.

2.3. Study endpoints and rationale

The primary endpoint will be to compare the absolute changes in LDL-C levels from the baseline to 16 weeks after treatment initiation among patients receiving EXB-CTM combination therapy, EXB monotherapy, CTM monotherapy, and placebo (Table 1). Recent studies reported that the serum and hepatic BA levels increase in patients with NASH and that the severity of NASH is related to a specific change in enterohepatic BA circulation.[6] As the inhibition of BA reabsorption from the intestine is suggested to possibly improve NASH, we will focus on LDL-C reduction associated with increased BA production. LDL-C is commonly used for assessing NAFLD. For instance, phase II studies of VK2809 and obeticholic acid used LDL-C as the primary endpoint,[15] and a phase IIa study on EXB monotherapy for NAFLD in the USA used LDL-C as the primary endpoint. The NAFLD guidelines recommend using anti-dyslipidemic drugs to treat NAFLD. Because reducing the LDL-C level may improve cholesterol accumulation in the liver and is expected to improve NAFLD, LDL-C was considered an appropriate endpoint for monitoring NAFLD. As CTM, which is proposed to be used in the present study, is a hypercholesterolemia treatment, and EXB is shown to reduce LDL-C in patients with chronic constipation, LDL-C is expected to be a sensitive surrogate for monitoring the efficacy of the EXB–CTM combination therapy.

As we aim to confirm the proof-of-concept (POC) in this phase IIa study, it is appropriate to monitor treatment efficacy using LDL-C as a surrogate marker that can be tested within a short period rather than histopathological assessment using a more invasive liver biopsy. The secondary endpoints include liver function and liver fat fraction assessments that will provide a comprehensive evaluation. After confirming the POC in this study, we plan to perform drug-dosing studies in the next phase, including liver biopsy analysis for determining the NASH status and hepatic fibrosis after long-term drug administration.

Our current secondary endpoint will determine the absolute change in the liver fat fraction and hepatic fibrosis, measured by MRI-PDFF and magnetic resonance elastography (MRE) according to

our previous method,[16, 17] respectively, from the baseline to 16 weeks after treatment initiation. The other parameters that will be monitored as secondary endpoints for the absolute changes between the baseline and 16 weeks after treatment initiation include alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (γ-GTP), high-density lipoprotein cholesterol (HDL-C), non-HDL-C, LDL-C/HDL-C ratio, and triglyceride. Other variables monitored will be adverse events (AEs), standard laboratory analysis results, physical examination, vital signs, and compliance rate. Physical assessments will be performed and evaluated at Yokohama City University, using standard procedures.

Table 1. Study endpoints

Primary endpoint	**Secondary endpoints**	**Exploratory endpoints**
<Efficacy endpoint>	**<Efficacy endpoint>**	**<Efficacy endpoint>**
・Absolute change in serum LDL-C from the baseline to week 16	・Absolute change in the liver fat fraction (%), as measured by MRI-PDFF, from the baseline to week 16	<Class; lipid> Change in the following from the baseline to week 16: serum lipid fraction[a] and apoprotein A1 or B.
	・Absolute change in hepatic fibrosis, as measured by MRE, from the baseline to week 16	<Class; Endocrine> Change in the following parameters from the baseline to week 16: serum c-peptide, plasma total GLP-1, plasma active GLP-1, and plasma adiponectin.
	・Change in the following parameters from the baseline to week 16: ALT, AST, γ-GTP, HDL-C, non-HDL-C, LDL-C/HDL-C ratio, and TG	<Class; Bile acid> Change in the following parameters from the baseline to week 16: serum C4, FGF-19, total bile acid, and fraction of bile acid and fecal bile acid (total and fraction)
		<Class; Inflammation> Change in the following parameter from the baseline to week 16: plasma CK18 fragment M30, endotoxin activity assay, and plasma LBP.
	<Safety endpoint>	<Class; fibrosis> Change in the following parameters from the baseline to week 16: serum type IV

		collagen 7s, PIIIP, hyaluronic acid, and TIMP-1
	・Incidence of adverse events	<Class; gut-microbiota> Change in fecal gut-microbiota from the baseline to week 16
	・Change in the daily score of the BSFS, number of bowel movements, and PAC-QOL from the baseline at each visit	<Class; other> Change in serum TMAO from the baseline to week 16
	・Change in the CLDQ scale from the baseline at each visit	

All objectives will be compared among the EXB (10 mg) and CTM (9 g) combination therapy, EXB (10 mg) or CTM (9 g) monotherapy, and placebo groups. ALT, alanine transaminase; AST, aspartate transaminase; BSFS, Bristol Stool Form Scale; CLDQ, Chronic Liver Disease Questionnaire; CTM, cholestyramine; EXB, elobixibat; FGF-19, fibroblast growth factor 19; γ-GTP, γ-glutamyl transpeptidase; GLP-1, glucagon-like peptide-1; HDL-C, high-density lipoprotein cholesterol; LBP, lipopolysaccharide-binding protein; LDL-C, low-density lipoprotein cholesterol; PAC-QOL, Patient Assessment of Constipation Quality of Life; PIIIP, procollagen-3-peptide; TIMP-1, tissue inhibitor of metalloproteinase-1; TMAO, trimethylamine N-oxide.

[a] chylomicron-cholesterol, chylomicron-TG, intermediate density lipoprotein cholesterol, very low-density lipoprotein cholesterol (VLDL-C), LDL-C, HDL-C, LDL-TG, VLDL-TG.

2.4. Rationale for treatment dose, mode, and duration

We considered it was appropriate to use the currently approved dose of each drug in our study to investigate the effects of the EXB–CTM combination therapy in an exploratory manner and provide an adequate and safe experience under clinical management. Although not previously published or presented at academic meetings, this combination therapy at the approved dosing is widely known to improve hepatic parameters and follow-up without adverse drug reactions, including diarrhea and constipation, in patients with NAFLD in clinical settings in Japan. The approved dosing should be sufficient to ensure safety and tolerability in patients with NAFLD and allow an efficacy evaluation of the combination therapy.

Although the hypercholesterolemia regimen is administered at 9 g/dose, 2–3 times daily, the dose in this study will be administered once daily before breakfast concomitant with EXB. This regimen is based on the consideration that a combination therapy is preferable for achieving continuous drug compliance, along with reducing AEs due to EXB, including abdominal pain and diarrhea.

Global exploratory NAFLD trials have adopted a treatment period of at least 12 weeks (Safety, Tolerability, Pharmacokinetics and Efficacy of LMB763 in Patients With NASH: NCT02913105; A Study of the Efficacy and Safety of CF102 in the Treatment of Non-Alcoholic Fatty Liver Disease: NCT02927314). A Japanese phase III study of EXB in patients with chronic constipation demonstrated that the LDL-C level rapidly decreased after 2 treatment weeks, and lower levels were maintained for at least 52 weeks.[7] However, in this study, we plan to use MRI examination as a secondary endpoint, and it requires a minimum treatment period for efficacy determination. Thus, a 16-week treatment period was incorporated into the study design to ensure an appropriate EXB efficacy evaluation.

2.5. Drug supply

Only the Patient Enrolment Centre will know the treatment allocation, and double-blinding of the physicians and patients will be maintained throughout the study until all patients have completed the 16-week study, and the database has been locked for all study data. Tablets of EXB (5 mg) and the corresponding reference placebo, which is indistinguishable in appearance, were manufactured and supplied by EA Pharma Co., Ltd. (Tokyo, Japan). The CTM powder, 9 g/dose (CTM 4 g), was purchased from the market, and its reference placebo, which is indistinguishable in appearance, was manufactured; both were supplied by TOYO Pharmaceutical Co., Ltd. (Osaka, Japan). For the study drugs prescribed, the physicians will enter the drug allocation number provided by the Patient Enrolment Centre on the prescription form. The drug manager will dispense the study drug to the patient with the drug allocation number. Exceptions to blinding will be to secure a patient's safety and for treatment upon request to the Patient Enrolment Centre personnel by the principal investigator.

2.6. Sample size estimation

Due to the exploratory nature of this study, formal power calculations were not used to determine sample sizes. The number of subjects (100 patients in total, 25 patients per treatment group) was chosen based on the data from a Japanese phase III clinical study and a long-term study on EXB in patients with chronic constipation.[7] The phase III study showed an LDL-C level reduction after 2 weeks of treatment, whereas the long-term study demonstrated that similar reductions persisted from week 4 to week 52 of treatment. Hence, we hypothesize that the reduction in the LDL-C level associated with EXB treatment (10 mg) will remain the same between weeks 2 and 16. As the mean difference in the LDL-C level from the baseline between the 10 mg EXB group and placebo group was -15.7 mg/dL, the pooled standard deviation was predicted as 17.9 mg/dL. Using sample sizes of $n = 20$ per group, the study has 80% power with two-sided $\alpha = 0.05$. Taking protocol violations and dropouts into account, a sample size of 25 patients in each group was calculated to compare EXB

and placebo groups. Assuming that there is no synergistic effect of EXB and CTM, the powers to compare (1) CTM use and CTM no-use groups, and (2) EXB+CTM and placebo groups will be large enough.

2.7. Eligibility

The physicians will enter legally capable patients into the Screening List, assign an identification code to each patient, and determine eligibility according to the inclusion and exclusion criteria (Table 2). If no eligibility issues are identified, the investigator or sub-investigator and investigative staff will enter the necessary information into the Electronic Data Capture (EDC) system for enrolment. The patient enrolment number will then be assigned, and enrolment will be completed.

Table 2. Patient inclusion and exclusion criteria

	Inclusion Criteria	**Exclusion Criteria**
1	Patients who received adequate explanation about this study and provided written informed consent	Women who are pregnant, breastfeeding, possibly pregnant, or do not agree to use birth control during the study
2	Patients who are ≥20 and <75 years of age at the time of informed consent	BMI <23 kg/m^2

3	Patients who have a current biopsy-confirmed NASH within 8 months of screening or a suspected diagnosis of NAFLD/NASH based on the criteria outlined below: (1) Biopsy-confirmed NASH is defined as histological NASH diagnosis with fibrosis stage F1 through F3 and a NAFLD activity score (NAS) of ≥4 with a score of ≥1 in each of the NAS components below as assessed by a pathologist using the NASH Clinical Research Network criteria: 1) Steatosis (scored 0–3) 2) Ballooning degeneration (scored 0–2) 3) Lobular inflammation (scored 0–3) (2) The suspected diagnosis of NAFLD/NASH is based on the following criteria: 1) AST ≥ 20 U/L and ALT ≥ 40 U/L in males or ≥ 28 U/L in females 2) Waist circumference ≥ 85 cm in males or ≥ 90 cm in females 3) Diagnosis of metabolic syndrome having 2 or more of the following 3 risk factors at screening: a): Fasting plasma glucose ≥ 110 mg/dL or undergoing drug treatment for elevated glucose b): SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg or undergoing drug treatment for hypertension, or antihypertensive drug treatment in a patient with a history of hypertension c): TGs ≥ 150 mg/dL or undergoing drug treatment for elevated TGss, and/or HDL-C < 40 mg/dL or undergoing drug treatment for reduced HDL-C	Liver stiffness measured by MRE >6.7 kPa

4	Screening MRI -PDFF with ≥8% liver steatosis	Any of the following laboratory abnormalities: (1) ALT >5 × ULN or AST >5 × ULN (2) PT-INR ≥1.3 unless on anticoagulant therapy (3) Total bilirubin > ULN, except with an established diagnosis of Gilbert's syndrome (4) Platelet count < 80,000/μL (5) eGFR <45 as calculated by the BSA adjustment (normalized eGFR)
5	Fasting serum LDL-C > 120 mg/dL or receiving antidyslipidemic drugs	Acute or chronic liver disease other than NAFLD/NASH including but not limited to the following: (1) Hepatitis B (as defined by the presence of HB antigen at screening) or hepatitis C (as defined by the presence of HCV antibody [anti-HCV]) Patients with positive anti-HCV who test negative for HCV ribonucleic acid (HCV-RNA) at screening will be allowed to participate in the study as long as there is evidence of viral negativity for a minimum of 12 months before screening (2) Evidence of AIH (3) History of PBC, PSC, Wilson's disease, alpha-1-anti-trypsin deficiency, hemochromatosis or iron overload, drug-induced ALD, or known bile duct obstruction (4) Suspected or proven HCC
6	Be willing to maintain a stable diet and physical activity throughout the course of the study	Known history of HIV
7		Medical history of liver cirrhosis
8		Clinical evidence of portal hypertension to include any history of ascites, hepatic encephalopathy, or presence of esophageal varices
9		Use of drugs historically associated with NAFLD (amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, or valproic acid) or other known hepatotoxins for ≥2 weeks in the year before Screening

10		Use of the following medications: (1) GLP-1 agonists unless on a stable dose 3 months before screening or liver biopsy (2) Ursodeoxycholic acid or thiazolidinediones within 3 months before screening (3) Antidyslipidemic drugs have been stable for ≥3 months before Screening (4) Oral antidiabetic drugs have been stable for ≥3 months before screening (5) Agents (including herbal over-the-counter weight loss preparations) or medications known to significantly affect body weight within 3 months before screening
11		History of significant alcohol consumption, defined as an average of ≥20 g/day in female patients and ≥30 g/day in male patients, for a period of >3 consecutive months within 1 year before screening, hazardous alcohol use (Alcohol Use Disorders Identification Test score ≥8), or an inability to reliably quantify alcohol consumption but determined as alcohol polydipsia based on the judgment of the investigator or sub-investigator
12		Weight change of ≥10% within the 6 months before screening or ≥5% within the 3 months before screening
13		Surgery planned during the study period or after bariatric surgery (e.g., gastroplasty and Roux-en-Y gastric bypass)
14		Type 1 diabetes by medical history
15		Uncontrolled type 2 diabetes defined as hemoglobin A1c (HbA1c) of >9.5% at screening (patients with HbA1c

		>9.5% may be rescreened) or requiring insulin dose adjustment of >10% within 2 months before screening
16		Clinical hyperthyroidism or hypothyroidism or screening hormone results pointing to thyroid dysfunction. Patients receiving dose-stable thyroid replacement therapy for ≥3 months before screening will be allowed to participate in this study as long as thyroid tests show that the patient is euthyroid and stable
17		History of any condition causing malabsorption such as chronic pancreatitis, extensive bowel/small intestine surgery, celiac disease, or bile flow obstruction
18		History of any condition associated with acute or chronic diarrhea such as IBD, functional diarrhea, IBS with predominant diarrhea, IBS with mixed bowel habits, or unclassified IBS
19		Uncontrolled hypertension (either treated or untreated) defined as SBP of >160 mmHg or a DBP of >100 mmHg at Screening
20		A history of New York Heart Association (NYHA) Class III or IV heart failure, or known left ventricular ejection fraction of <30%
21		A history of myocardial infarction, unstable angina, percutaneous coronary intervention, coronary artery bypass graft, or stroke or major surgery within 6 months prior before screening
22		Active substance abuse, within 1 year before screening
23		Participation in an investigational new drug trial in the 30 days before screening or within 5 half-lives of an investigational agent, whichever is longer
24		Complication with malignancy Patients with a history of malignancies that have been treated with a curative intent or completed chemotherapy may be eligible Patients under evaluation for malignancy are not eligible

25		Known intolerance to MRI or conditions contraindicated for MRI procedures
26		Any other condition which is considered to be inappropriate for the study by the Investigator or sub-investigator

AIH, autoimmune hepatitis; ALD, alcoholic liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BSA, body surface area; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide-1; HBs, hepatitis B surface; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDL-C, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; LDL-C, low-density lipoprotein cholesterol; MRE, magnetic resonance elastography; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; PDFF, proton density fat fraction; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; PT-INR, prothrombin time-international normalized ratio; SBP, systolic blood pressure. TG, triglyceride, ULN, upper limit normal.

2.8. Randomization and masking

The patients will be randomized to each group (EXB+CTM, EXB, CTM, and PBO) at a ratio of 1:1:1:1 using a computer-generated centrally administered procedure. The applicable drug number will be assigned via the EDC. All trial drugs will be packaged identically and identified only by the number assigned. As noted above, the treatment assignments will be fully masked from the patients and physicians.

2.9. Keycode break

If the investigator or sub-investigator considers it urgently necessary to break the study keycode prematurely, they will contact the person responsible for study drug randomization to file the request. This may occur due to a serious AE (SAE), the need for treating an AE, or a similar situation.

2.10. Harms and AE monitoring

AEs are defined as any unfavorable or unintended sign (including laboratory parameters and abnormal vital signs), symptom, or disease that may occur during the study period. AEs that are not directly related to the study drugs may develop. The investigator or sub-investigator will assess the severity of the AEs. Any AE that meets any of the following criteria will be considered an SAE: death, life-threatening, hospitalization requirement or prolonged hospitalization for treatment,

disability, disability threat, other serious conditions, congenital disease, or anomaly in offspring. If an SAE occurs, the investigator or sub-investigator will appropriately treat it, and the investigator will immediately report the details to the Hospital Director and the study drug supplier.

2.11. Study procedures

The investigator or sub-investigator will perform all observations, tests, investigations, and evaluations according to the descriptions provided in Table 3. If a blood test is scheduled at a study visit, blood will be collected only after the patient had fasted for at least 8 h.

Table 3. Schedule of observations, tests, and assessments

	Before Screening	Screening	Treatment Period				Follow-Up
		V1	V2/randomization	V3	V4	V5	V6/EOT	V7
Study week		Week -8 to day -1	Day -1	Week 4	Week 8	Week 12	Week 16	EOT to 2 weeks
Visit window			-	±7 days	±7 days	±7 days	±7 days	±7 days
Informed consent	○							
Inclusion/exclusion criteria		○	○					
Demographics		○						
Serology [a]		○						
Chest X-ray		○						
ECG		○						
Physical examination [b]		○	○				○	
Vital signs [c]		○	○	○	○	○	○	○
Pregnancy test [d]		○	○				○	
MRI [e]		○					○	
Liver biopsy		△						
Randomization			○					
Hematology/urinalysis [f]		○	○[k]	○	○	○	○	○
Endocrinology		○						

Biochemistry 2 [g]		○	○[k]				○	
Total bile acids			○[k]				○	
Lipid profile		○	○[k]	○	○	○	○	○
Others [h]			○[k]				○	
Blood/stool sampling for storage			●				●	
Dispense study drug			○	○	○	○		
Review study drug compliance				○	○	○	○	
Review alcohol consumption [i]		○						
Review concomitant medications		○	○	○	○	○	○	
Review adverse events		○	○	○	○	○	○	○
Questionnaire/review patient diary [j]			○	○	○	○	○	

○: To be performed

△: Information will be collected from patients who have liver biopsy results.

●: Blood/stool sampling for storage will be collected for the analysis of genes, fibrosis, and inflammation, upon obtaining consent separately.

[a]: Includes HB antigen, HCV antibody, and HCV-RNA.

[b]: Includes height (at V1 only), body weight, waist circumference, and waist-to-hip ratio. BMI will be calculated based on height and weight.

[c]: Vital signs include blood pressure, heart rate, respiratory rate, and axillary temperature.

[d]: For women of childbearing potential, a urinary pregnancy test will be performed at V1, V2, and V6. The test is not required at V2 when it occurs within 1 month after V1.

[e]: Patients will undergo an MRI to measure liver fat (PDFF) and total liver volume. Patients who discontinue before V6 (week 16) should undergo an MRI at the end of treatment if they completed at least 4 weeks of treatment.

[f]: Hematology/urinalysis includes hematology/coagulation, biochemistry 1, and urinalysis.

[g]: Biochemistry 2 includes glucose, HbA1c, and insulin.

[h]: Others include high-sensitivity CRP, type IV collagen 7s, FIB-4, and APRI.

[i]: History of alcohol consumption will be obtained at screening.

[j]: Questionnaires of PA-QOL and CLDQ will be answered at each visit. Number of bowel movements and BS score will be collected from the patient diary.

[k]: Data within 1 month can be used as a substitute.

2.12. Criteria and procedure for withdrawal from the study

The investigator or sub-investigator will discontinue the study enrollment of a patient if the participant meets any of the following criteria: (1) the patient desires withdrawal, (2) after enrollment, the patient is found not to meet the inclusion criteria or exclusion criteria, (3) if the investigator or sub-investigator opines that having the patient continue in the study is not appropriate due to an AE, and (4) if the investigator or sub-investigator opines that having the patient continue in the study is not appropriate due to any other reason.

2.13. Efficacy evaluation

The primary efficacy endpoint will be the absolute change in LDL-C level from the baseline to 16 weeks after treatment initiation. The secondary and other efficacy endpoints are provided in Table 1. The assessment of the MRI-PDFF/MRE will be performed by an independent liver specialist who is blinded to the treatment (IK).

2.14. Safety assessments

The following safety evaluations will be performed during each patient visit from the time of treatment initiation until the 2-week follow-up period: (1) incidence of AEs in the treatment groups compared with that of the placebo groups, (2) change in the daily score of the Bristol Stool Form Scale from the baseline regarding the number of bowel movements and the Patient Assessment of Constipation Quality of Life (PAC-QOL), (3) change in the Chronic Liver Disease Questionnaire (CLDQ) scale from the baseline.

2.15. Population analysis

The set of subjects to be analyzed will be determined before locking the data of each patient and will be defined as follows. The modified intention-to-treat, which is the full analysis set (FAS), and per-protocol set (PPS) will be used for the assessment of primary efficacy. The FAS will include all patients who are randomized, except those who meet any of the following criteria: (1) patients with major protocol deviation (e.g., deviation of informed consent and major deviation in the study procedures), (2) patients who have not received any dose of the study drugs, and (3) patients who have no measurement of the efficacy endpoint. A PPS will include patients without protocol deviations. The Safety Analysis Set (SAS) will be used for safety assessment and will include all patients who receive at least one dose of the study drug.

2.16. Statistical analysis

Considering the placebo-controlled randomized parallel-group comparison design, we will use descriptive statistics to summarize differences between the placebo group and each monotherapy or combination group and those between each monotherapy group and the combination group. Unless otherwise noted, the descriptive statistics include the number of patients, mean, standard deviation, median, minimum and maximum values of continuous variables, frequency, and percentage of categorical variables.

For the primary endpoint, the change in serum LDL-C from the baseline to week 16 (difference in measurement values between time points) will be summarized for each treatment group. Point estimates and confidence intervals for the mean difference from the baseline between the placebo group and each monotherapy or combination group and those between each monotherapy group and the combination group will be calculated. The changes over time in the measured parameters will be summarized for each treatment group. The ratio of change (ratio of the absolute change to the baseline value) will also be analyzed using the same methods. The secondary endpoints will be analyzed similar to the primary endpoint. Due to the exploratory nature of this study, a concrete statistical hypothesis is not formulated. For exploratory purposes, in case any statistical test is performed or confidence interval of any parameter is calculated, the significance level of 5% (two-sided) and the confidence coefficient of 95% will be used. Multiplicity arising from the interpretation of statistical tests or confidence intervals will not be adjusted.

2.17. Interim analysis

Not applicable.

2.18. Data management, central monitoring, and audit

The investigators' sites will maintain individual records of each patient as source data, which include a copy of informed consent, medical records, laboratory data, and other records or notes. All data will be collected by the independent data management center. The data management center will oversee the interstudy data sharing process. The clinical data entry, data management, and central monitoring will be performed using the electric data capture VIEDOC 4 (PCG Solutions, Sweden). Furthermore, auditing will be planned at an external clinical research organization.

2.19. Efficacy and safety assessment board

Although no interim analysis will be performed in this study, this committee was established to monitor the overall study focusing on safety, as this is the first study to administer elobixibat and

cholestyramine in combination to patients with NAFLD. The board will consist of three external board members who have no relationship with the operations involved in the study.

2.20. Study flow and schedule of enrolment, interventions, and assessments

A study flowchart is shown in Fig. 1. The study schedule is presented in Table 3.

2.21. Patient and public involvement

In this randomized controlled trial, patients will be involved in the recruitment to and conduct of the study. Especially, the development of the research question and outcome measures were based on patients' priorities, experience, and preferences. The results of this study will be disseminated by email to the participants who indicate that they want the results. The burden of intervention will be assessed by patients before the commencement of the trial; patients' satisfaction of the treatment will be collected as a part of the post-intervention assessment.

3. DISCUSSION

This is the first proof-of-concept study proposed to explore the effect of EXB in combination with CTM in patients with NAFLD. A previous study showed that an IBATi improved both hepatic and whole-body aspects in a mouse model of NAFLD.[9] However, EXB causes AEs, including abdominal pain and diarrhea, by increasing colonic BAs, resulting in the development of BA-induced diarrhea. Therefore, the safety and tolerability of monotherapy with EXB might decrease in patients with NAFLD. Considering the MOA, the removal of excess BAs from the colon using CTM has the potential to improve BA-induced diarrhea.

In some well-known trials (e.g., PIVENS, FLINT, and GOLDEN),[15, 18-20] the primary endpoints included liver histology, which was evaluated using liver biopsy specimens. Liver histology endpoints, such as the complete resolution of NASH, are considered surrogates for preventing cirrhosis because they are considered predictive of clinical benefit but are not direct measures. Presently, as described in the guidelines ("the gold standard"), the only way to diagnose NASH is via a liver biopsy. However, performing liver biopsies in all patients with NAFLD is difficult as there are more than 10 million patients with NAFLD in Japan. In addition, there are various problems associated with liver biopsies, such as invasiveness, cost, and misinterpretation between pathologists.

Recently, significant advances have been made in MRI techniques, and hepatic steatosis and fibrosis can be diagnosed at extremely high sensitivity and specificity using MRE.[21, 22] It is also possible to quantify the fat ratio using MRI by measuring the PDFF using the iterative decomposition of water

and fat with echo asymmetry and least-squares estimation sequence (IDEAL IQ) method.[17, 23] MRE and MRI-PDFF can be performed simultaneously in one imaging session, and by combining the results, hepatic steatosis and hepatic fibrosis can be evaluated. MRI-based noninvasive assessment of liver fibrosis and steatosis would be a potential alternative to liver biopsy in clinical practice.[17] Hence, we chose MRI for the noninvasive assessment of hepatic steatosis in this study. Quantification of liver fat content using MRI-PDFF has been previously shown to be sensitive in detecting changes, and it has been used in nonalcoholic steatohepatitis clinical trials for quantitative fat assessment.[17, 21] Using paired MRI-PDFF and liver histology data, Patel et al. have shown that an absolute change in liver fat content of -4.1% with a relative change of -29.3% associated with histological improvements, that is, reduction in the NAFLD Activity Score (NAS) by two or more points, with 1 point each for steatosis and ballooning.[16] This suggests that changes in liver fat may also be a surrogate for early hepatocellular injury. Furthermore, weight-loss studies have demonstrated a clinically meaningful decrease of 4.7%–4.8% in the absolute liver fat content between the beginning and end of the intervention.[24] Based on these considerations, and consistent with recent trials in the field, we chose ≥5% reduction in the absolute liver fat content as a responder criterion for primary outcome and a relative 30% reduction as a clinically meaningful change in the liver fat content.

Our study has the following strengths: (1) the first randomized, placebo-controlled, double-blinded study focused on removing BAs in patients with NAFLD; (2) a novel POC study considering the drug MOA for improving the treatment effect and tolerability, as well as reducing the AEs; (3) MR images will be captured following a standardized protocol and processed under the supervision of a hepato-radiologist blinded to the study; (4) the comparison of colocalized regions of interest for fat content changes in each of the nine liver segments between weeks 0 and 16; (5) the final assessment of the MRI examinations will be randomized with regards to time points in order to reduce bias; and (6) the measurement of exploratory endpoints such as the lipid class, endocrine functions, BA, inflammation, fibrosis, gut-microbiota, and trimethylamine-N-oxide (Table 2). Nevertheless, our study also has the following limitations: (1) will be performed in a single center; (2) a relatively small sample size; (3) a relatively short treatment duration; and (4) the lack of liver biopsy.

NAFLD/NASH is a heterogeneous disease with correspondingly complex pathophysiology, which includes redundant pathways that may not be uniform among patients. Considering the MOA of pharmaceuticals for treating NAFLD/NASH, its complex pathophysiology permits the development of a wide array of potentially viable therapeutic targets, especially components of anti-metabolic, anti-inflammatory, and anti-fibrotic pathways.[25] In a phase IIa trial for NASH/NAFLD therapies, BMS-986036 (FGF21) [26] and NGM282 (FGF19) [27] exerted an anti-metabolic effect, and the liver fat content was used as the primary endpoint. Although the current NAFLD therapy focuses on anti-metabolic, anti-inflammatory, and anti-fibrotic aspects in the liver as MOA, decreasing BAs for

treating NAFLD is a novel therapeutic target for patients with NAFLD. EXB–CTM combination therapy-associated improvements in the changes of the absolute and relative liver fat levels changes are clinically relevant.

DECLARATIONS

Ethics approval and consent to participate

The Ethics Committee of Yokohama City University Hospital approved the study protocol on November 26, 2019. This trial is registered with ClinicalTrials.gov (number NCT04235205). Informed consent will be obtained from all participants before enrolment. Any information supplied to obtain informed consent was also approved by the Ethics Committee.

confidentially

Data will be retained in accordance with the Japanese ethical guidelines for clinical research. Participants will be allocated a unique identification (ID) number at entry. The master list linking participant personal information and ID number will be maintained in a separate locked cabinet and password-protected hard drive. Data will be analyzed by ID number only. Records will be retained for 5 years after study completion, and then destroyed by the data center.

Consent for publication and dissemination

The results of this study will be submitted for publication in international peer-reviewed journals and the key findings will be presented at conferences. Participants will be informed of the results of the trial by the investigators. Authorship will be ascribed in accordance with the International Committee of Medical Journal Editors guidance.

Competing interests

AN reports grants and research support from Gilead, Mylan EPD, EA Pharma, Kowa, Taisho, Biofermin; is a consulting adviser for Gilead, Boehringer Ingelheim, BMS, Kowa, Astellas, EA Pharma, Mylan EPD. Other authors declare no competing interests.

Funding

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Authors' contributions

TKessoku and AN participated in study design. TKessoku and TKobayashi conducted feasibility

phase work. Recruitment of participants and follow-up will be performed by TKessoku, AO, MI, TKobayashi, YH, YO, MY, and SS. Reading of MRI will be done by KI. Exploratory item will be measured by HU and KW. TKessoku, TKobayashi, YS, KY, TY, and AN will participate in data interpretation. All authors contributed to writing, and all read and approved the final manuscript.

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Data sharing statement

Data are available upon reasonable request.

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FIGURE LEGEND

Figure 1. Study design. [a]N = 100 enrolled. CTM, cholestyramine; EXB, elobixibat; NAFLD, nonalcoholic fatty liver disease; PBO, placebo; QD, quaque die.

Figure 1

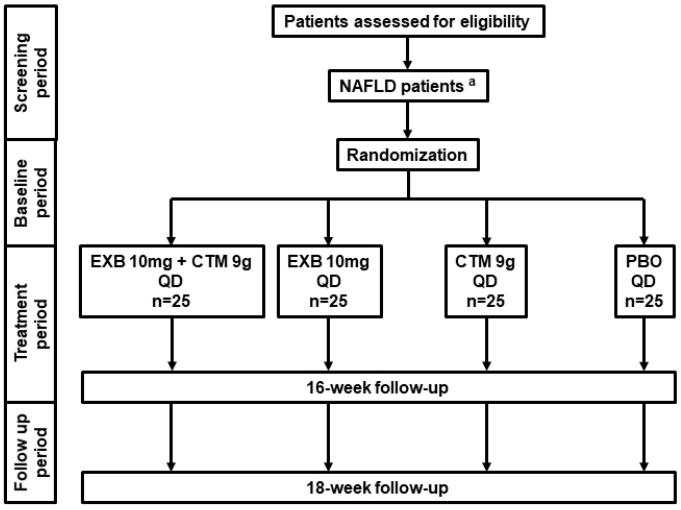

Figure 1

254x190mm (96 x 96 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2,5,6
	2b	All items from the World Health Organization Trial Registration Data Set	5,6
Protocol version	3	Date and version identifier	2
Funding	4	Sources and types of financial, material, and other support	23
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,23
	5b	Name and contact information for the trial sponsor	23
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	23
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	6,23
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4,5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10-16
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	21
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	19
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	17,18
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10-16
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6-8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	17-19, Fig.1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9,10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9,10
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	16,17
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	16,17

Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	16,17
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	16,17
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	16,17
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	17-19
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	17-19
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	19-21
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	20-21
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	20-21
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	20-21
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	17
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	21
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17

Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	17-18
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	5,6,23
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	23
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	23
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	23
	31b	Authorship eligibility guidelines and any intended use of professional writers	23
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Rationale and design of a randomized, double-blind, placebo-controlled, parallel-group, investigator-initiated phase 2a study to investigate the efficacy and safety of elobixibat in combination with cholestyramine for nonalcoholic fatty liver disease

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Rationale and design of a randomized, double-blind, placebo-controlled, parallel-group, investigator-initiated phase 2a study to investigate the efficacy and safety of elobixibat in combination with cholestyramine for nonalcoholic fatty liver disease

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Study dates: November 1, 2019 to December 31, 2020.

ABSTRACT

Introduction: Nonalcoholic fatty liver disease (NAFLD) pathogenesis involves abnormal metabolism of cholesterol and hepatic accumulation of toxic free-cholesterol. Elobixibat (EXB) inhibits the ileal bile acid (BA) transporter. EXB and cholestyramine (CTM) facilitate the removal of free cholesterol from the liver by decreasing BA recirculation to the liver, thereby stimulating novel BA synthesis from cholesterol. In this randomized, double-blind, placebo-controlled, parallel-group, phase IIa study, we aim to provide a proof-of-concept assessment by evaluating the efficacy and safety of EXB in combination with CTM in patients with NAFLD.

Methods and analysis: A total of 100 adult patients with NAFLD, diagnosed based on low-density lipoprotein cholesterol (LDL-C) level of >120 mg/dL and liver fat content of ≥8% by magnetic resonance imaging (MRI)-based proton density fat fraction (MRI-PDFF), who meet the inclusion/exclusion criteria will be enrolled. The patients will be randomly assigned to receive the combination therapy of 10 mg EXB and 9 g CTM powder (4 g CTM), 10 mg EXB monotherapy, 9 g CTM powder monotherapy, or a placebo treatment (n = 25 per group). Blood tests and MRIs will be performed 16 weeks following treatment initiation. The primary study endpoint will be the absolute LDL-C level change at week 16 after treatment initiation. The exploratory endpoint will include absolute changes in the liver fat fraction as measured by MRI-PDFF. This proof-of-concept study will determine whether the combination therapy of EXB and CTM is effective and safe for patients with NAFLD.

Ethics and dissemination: Ethics approval was obtained from the Ethics Committee of Yokohama City University Hospital before participant enrollment. The results of this study will be submitted for publication in international peer-reviewed journals and the key findings will be presented at international scientific conferences.

Trial registration: This trial is registered with ClinicalTrials.gov (number NCT04235205).

Protocol version: 1.3, February 20, 2020

Keywords: Nonalcoholic fatty liver disease, elobixibat, cholestyramine, combination therapy, low-density lipoprotein cholesterol, phase 2a

Strengths and limitations of this study

- This study is the first randomized, double-blind, placebo-controlled, phase 2a trial to determine the efficacy and safety of elobixibat in combination with cholestyramine for nonalcoholic fatty liver disease
- This original proof-of-concept study aims to explore the additive effects and the reduction in the adverse events following combination therapy, compared to those of monotherapy.

- The primary outcome is the improvement in low-density lipoprotein cholesterol, and the exploratory outcome is liver fat content assessed by magnetic resonance imaging-proton density fat fraction.
- Limitations are short treatment duration and the lack of liver biopsy.

INTRODUCTION[1]

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[1]**Abbreviations**

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FAS, full analysis set; γ-GTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MOA, mechanism of action; MRI-PDFF, magnetic resonance imaging-

Nonalcoholic fatty liver disease (NAFLD) is a clinical condition that is detected by tissue or image analyses and diagnosed by excluding alcoholism and other liver diseases. It is the hepatic manifestation of a metabolic syndrome and is often associated with obesity, diabetes mellitus, dyslipidemia, hypertension, and other disorders. The prevalence of NAFLD is increasing worldwide; in Japan, it increased from 12.9% in 1994 to approximately 34.7% in 2000.[1] NAFLD is classified as nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH), which includes inflammation and a progressive disease associated with liver cancer or cirrhosis, affecting 10%–20% of the patients.[2] The factors contributing to NASH pathogenesis are abnormal cholesterol metabolism and free cholesterol accumulation in the liver, which is toxic to hepatocytes, leads to inflammation and fibrosis.[3]

Bile acids (BAs) may be critical in NASH pathogenesis.[4-6] They act as signaling molecules in lipid, glucose, and energy homeostasis, whose metabolic pathways are linked to NAFLD/NASH and comorbidities, including metabolic syndrome, obesity, and diabetes. The BAs and free cholesterol, as the precursor for BA synthesis, can act as lipotoxic agents that drive inflammation and fibrosis. In previous studies, both serum and liver BA levels were elevated in patients with NASH, and recent data suggested that the occurrence and severity of NASH are associated with specific changes in circulating BAs.[6] Therefore, targeting the BA pathways may have a therapeutic potential for patients with NAFLD.

Elobixibat (EXB) inhibits the ileal BA transporter (IBAT) and is approved for marketing as an oral treatment for chronic constipation.[7] The IBAT, expressed primarily in the distal ileum, is a key element in the enterohepatic circulation of BAs, as it facilitates BA reabsorption. EXB is orally administered and acts locally in the gut, where it reversibly binds to IBAT to decrease the re-uptake of BAs to the liver. EXB at expected therapeutic doses has minimal systemic exposure. The pharmacodynamic assessment of EXB in a Japanese phase I study showed decreased serum LDL-C levels in groups that received repeated doses of ≥5 mg EXB.[8] In addition, a study on NAFLD mouse model reported that treatment with another IBAT inhibitor (IBATi) promoted BA biosynthesis from cholesterol, which accumulated in the liver of mice that received a high-fat diet.[9] Furthermore, converting the BA pool in the liver into hydrophobic BAs decreases hepatic cholesterol accumulation via farnesoid X receptor (FXR) activation, thereby improves the NAFLD status.[9] EXB is also expected to improve the NAFLD status as it has the same mechanism of action (MOA) and lowers the LDL-C levels. However, increased BA level in the large intestine may stimulate the secretion of water and electrolytes in the intestinal tract and movement of the

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based proton density fat fraction; MRE, magnetic resonance elastography; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; POC, proof-of-concept; SAS, safety analysis set.

gastrointestinal tract, which induces BA diarrhea and promotes peristalsis.[10, 11]

The BA sequestrant (BAS) cholestyramine (CTM) is a strong base anion exchange resin, and its drug product alleviates hypercholesteremia and eliminates active leflunomide metabolites from the body. CTM inhibits the absorption of exogenous cholesterol by binding to BA in the intestinal tract, which increases feces excretion. CTM also promotes cholesterol catabolism to BA in the liver to compensate for the decreased BA level due to increased excretion. This ultimately decreases the LDL-C level. These effects are expected to promote hepatic lipid metabolism. In a Japanese clinical study, colesevelam, another BAS, improved fatty liver and liver enzymes in patients with NASH.[12] However, the improvement in fatty liver with colesevelam treatment is not consistently recognized as an outcome because it has not been demonstrated in other studies.[13] In addition to LDL-C reduction, colesevelam can alleviate BA diarrhea,[14] and a combination therapy of IBATi and BAS may prevent diarrhea, an adverse effect of IBATi. Our study aims to investigate the efficacy and safety of EXB in combination with CTM in patients with NAFLD.

MATERIALS AND METHODS

Trial design

The Standard Protocol Items for Randomized Trials (SPIRIT) statement and its checklist were followed in preparing the study protocol. This trial is designed as a single-center, randomized, double-blind, placebo-controlled, parallel-group, investigator-initiated study to investigate the efficacy and safety of a combination therapy of 10 mg EXB tablet and 9 g CTM powder formulation (4 g CTM), an EXB monotherapy (10 mg), or a CTM monotherapy CTM (9 g), compared with those of placebo treatments. All treatments will be administered orally once daily for 16 weeks to patients with NAFLD. The experimental groups will be as follows (Fig. 1): the EXB+CTM group (10 mg EXB and 9 g CTM), the EXB group (10 mg EXB and CTM placebo), the CTM group (EXB placebo and 9 g CTM), and the PBO group (EXB placebo and CTM placebo). Magnetic resonance imaging (MRI) will be performed at the baseline and 16 weeks after intervention and the data will be evaluated by a blinded independent liver specialist (KI). The study plan involves recruiting 100 adult patients with NAFLD from the Yokohama City University Hospital cohort.

Study endpoints and rationale

The primary endpoint will be to compare the absolute changes in LDL-C levels from the baseline to 16 weeks after treatment initiation among patients receiving EXB-CTM combination therapy, EXB monotherapy, CTM monotherapy, and placebo (Table 1). Recent studies reported that the serum and hepatic BA levels increase in patients with NASH and that the severity of NASH is related to a specific change in enterohepatic BA circulation.[6] As the inhibition of BA reabsorption from the intestine is suggested to possibly improve NASH, we will focus on LDL-C reduction associated with

increased BA production. LDL-C is commonly used for assessing NAFLD. For instance, phase II studies of VK2809 and obeticholic acid used LDL-C as the primary endpoint,[15] and a phase IIa study on EXB monotherapy for NAFLD in the USA used LDL-C as the primary endpoint. The NAFLD guidelines recommend using anti-dyslipidemic drugs to treat NAFLD. Because reducing the LDL-C level may improve cholesterol accumulation in the liver and is expected to improve NAFLD, LDL-C was considered an appropriate endpoint for monitoring NAFLD. As CTM, which is proposed to be used in the present study, is a hypercholesterolemia treatment, and EXB is shown to reduce LDL-C in patients with chronic constipation, LDL-C is expected to be a sensitive surrogate for monitoring the efficacy of the EXB–CTM combination therapy.

As we aim to confirm the proof-of-concept (POC) in this phase IIa study, it is appropriate to monitor treatment efficacy using LDL-C as a surrogate marker that can be tested within a short period rather than histopathological assessment using a more invasive liver biopsy. The exploratory endpoints include liver function and liver fat fraction assessments that will provide a comprehensive evaluation. After confirming the POC in this study, we plan to perform drug-dosing studies in the next phase, including liver biopsy analysis for determining the NASH status and hepatic fibrosis after long-term drug administration.

Our current exploratory endpoint will determine the absolute change in the liver fat fraction and hepatic fibrosis, measured by MRI-PDFF and magnetic resonance elastography (MRE) according to our previous method,[16, 17] respectively, from the baseline to 16 weeks after treatment initiation. The other parameters that will be monitored as exploratory endpoints for the absolute changes between the baseline and 16 weeks after treatment initiation include alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (γ-GTP), high-density lipoprotein cholesterol (HDL-C), non-HDL-C, LDL-C/HDL-C ratio, and triglyceride. Other variables monitored will be adverse events (AEs), standard laboratory analysis results, physical examination, vital signs, and compliance rate. Physical assessments will be performed and evaluated at Yokohama City University, using standard procedures.

Table 1. Study endpoints

Primary endpoint	**Exploratory endpoints**	
<Efficacy endpoint>	**<Efficacy endpoint>**	**<Safety endpoint>**
・Absolute change from baseline in serum LDL-C at Week 16	・Absolute change from baseline to Week 16 in liver fat fraction (%) as measured by MRI-PDFF	・Incidence of adverse events
	・Absolute change from baseline to Week 16 in hepatic fibrosis as measured by MRE	・Change from baseline in daily BSFS score, number of bowel

		movements, and PAC-QOL at every visit
	・Change from baseline to Week 16 in the following: ALT, AST, GGT, HDL-C, non-HDL-C, LDL-C/HDL-C ratio, and TG <Class; lipid> Change from baseline to Week 16 in the following: serum lipid fraction[a], apoprotein A1 or B. <Class; Endocrine> Change from baseline to Week 16 in the following: serum c-peptide, plasma total GLP-1, plasma active GLP-1 and plasma adiponectin. <Class; Bile acid> Change from baseline to Week 16 in the following: serum C4, FGF-19, total bile acid, and fraction of bile acid and fecal bile acid (total and fraction) <Class; Inflammation> Change from baseline to Week 16 in the following: plasma CK18 fragment M30, endotoxin activity assay, and plasma LBP <Class; fibrosis> Change from baseline to Week 16 in the following: serum type IV collagen 7s, PIIIP, hyaluronic acid, and TIMP-1 <Class; gut-microbiota> Change from baseline to Week 16 in fecal gut-microbiota <Class; other> ・Change from baseline to Week 16 in serum/fecal choline, trimethylamine, TMAO, amino acids. ・Change from baseline to Week 16 in fecal short chain fatty acids.	・Change from baseline in the CLDQ scale at every visit

All objectives will be compared among the EXB (10 mg) and CTM (9 g) combination therapy, EXB (10 mg) or CTM (9 g) monotherapy, and placebo groups. ALT, alanine transaminase; AST, aspartate transaminase; BSFS, Bristol Stool Form Scale; CLDQ, Chronic Liver Disease Questionnaire; CTM, cholestyramine; EXB, elobixibat; FGF-19, fibroblast growth factor 19; γ-GTP, γ-glutamyl transpeptidase; GLP-1, glucagon-like peptide-1; HDL-C, high-density lipoprotein cholesterol; LBP, lipopolysaccharide-binding protein; LDL-C, low-density lipoprotein cholesterol; PAC-QOL, Patient Assessment of Constipation Quality of Life; PIIIP, procollagen-3-peptide; TIMP-1, tissue inhibitor of metalloproteinase-1; TMAO, trimethylamine N-oxide.

[a] chylomicron-cholesterol, chylomicron-TG, intermediate density lipoprotein cholesterol, very low-density lipoprotein cholesterol (VLDL-C), LDL-C, HDL-C, LDL-TG, VLDL-TG.

Rationale for treatment dose, mode, and duration

We considered it was appropriate to use the currently approved dose of each drug in our study to investigate the effects of the EXB–CTM combination therapy in an exploratory manner and provide an adequate and safe experience under clinical management. Although not previously published or presented at academic meetings, this combination therapy at the approved dosing is widely known to improve hepatic parameters and follow-up without adverse drug reactions, including diarrhea and constipation, in patients with NAFLD in clinical settings in Japan. The approved dosing should be sufficient to ensure safety and tolerability in patients with NAFLD and allow an efficacy evaluation of the combination therapy.

Although the hypercholesterolemia regimen is administered at 9 g/dose, 2–3 times daily, the dose in this study will be administered once daily before breakfast concomitant with EXB. This regimen is based on the consideration that a combination therapy is preferable for achieving continuous drug compliance, along with reducing AEs due to EXB, including abdominal pain and diarrhea.

Global exploratory NAFLD trials have adopted a treatment period of at least 12 weeks (Safety, Tolerability, Pharmacokinetics and Efficacy of LMB763 in Patients With NASH: NCT02913105; A Study of the Efficacy and Safety of CF102 in the Treatment of Non-Alcoholic Fatty Liver Disease: NCT02927314). A Japanese phase III study of EXB in patients with chronic constipation demonstrated that the LDL-C level rapidly decreased after 2 treatment weeks, and lower levels were maintained for at least 52 weeks.[7] However, in this study, we plan to use MRI examination as an exploratory endpoint, and it requires a minimum treatment period for efficacy determination. Thus, a 16-week treatment period was incorporated into the study design to ensure an appropriate EXB efficacy evaluation.

Drug supply

Only the Patient Enrolment Centre will know the treatment allocation, and double-blinding of the physicians and patients will be maintained throughout the study until all patients have completed the 16-week study, and the database has been locked for all study data. Tablets of EXB (5 mg) and the corresponding reference placebo, which is indistinguishable in appearance, were manufactured and supplied by EA Pharma Co., Ltd. (Tokyo, Japan). The CTM powder, 9 g/dose (CTM 4 g), was purchased from the market, and its reference placebo, which is indistinguishable in appearance, was manufactured; both were supplied by TOYO Pharmaceutical Co., Ltd. (Osaka, Japan). For the study drugs prescribed, the physicians will enter the drug allocation number provided by the Patient Enrolment Centre on the prescription form. The drug manager will dispense the study drug to the patient with the drug allocation number. Exceptions to blinding will be to secure a patient's safety and for treatment upon request to the Patient Enrolment Centre personnel by the principal investigator.

Sample size estimation

Due to the exploratory nature of this study, formal power calculations were not used to determine sample sizes. The number of subjects (100 patients in total, 25 patients per treatment group) was chosen based on the data from a Japanese phase III clinical study and a long-term study on EXB in patients with chronic constipation.[7] The phase III study showed an LDL-C level reduction after 2 weeks of treatment, whereas the long-term study demonstrated that similar reductions persisted from week 4 to week 52 of treatment. Hence, we hypothesize that the reduction in the LDL-C level associated with EXB treatment (10 mg) will remain the same between weeks 2 and 16. As the mean difference in the LDL-C level from the baseline between the 10 mg EXB group and placebo group was -15.7 mg/dL, the pooled standard deviation was predicted as 17.9 mg/dL. Using sample sizes of $n = 20$ per group, the study has 80% power with two-sided $\alpha = 0.05$. Taking protocol violations and dropouts into account, a sample size of 25 patients in each group was calculated to compare EXB and placebo groups. Assuming that there is no synergistic effect of EXB and CTM, the powers to compare (1) CTM use and CTM no-use groups, and (2) EXB+CTM and placebo groups will be large enough.

Eligibility

The physicians will enter legally capable patients into the Screening List, assign an identification code to each patient, and determine eligibility according to the inclusion and exclusion criteria (Table 2). We will include only patients aged ≥20 and <75 years after obtaining informed consent. This is because (1) the legal adult age to obtain consent is 20 years in Japan and (2) patients over 75 years of age generally have impaired physiological function and are more prone to adverse events. If no eligibility issues are identified, the investigator or sub-investigator and investigative staff will

enter the necessary information into the Electronic Data Capture (EDC) system for enrolment. The patient enrolment number will then be assigned, and enrolment will be completed.

Table 2. Patient inclusion and exclusion criteria

	Inclusion Criteria	**Exclusion Criteria**
1	Patients who received adequate explanation about this study and provided written informed consent	Women who are pregnant, breastfeeding, possibly pregnant, or do not agree to use birth control during the study
2	Patients who are ≥20 and <75 years of age at the time of informed consent	BMI <23 kg/m^2
3	Patients who have a current biopsy-confirmed NASH within 8 months of screening or a suspected diagnosis of NAFLD/NASH based on the criteria outlined below: (1) Biopsy-confirmed NASH is defined as histological NASH diagnosis with fibrosis stage F1 through F3 and a NAFLD activity score (NAS) of ≥4 with a score of ≥1 in each of the NAS components below as assessed by a pathologist using the NASH Clinical Research Network criteria: 1) Steatosis (scored 0–3) 2) Ballooning degeneration (scored 0–2) 3) Lobular inflammation (scored 0–3) (2) The suspected diagnosis of NAFLD/NASH is based on the following criteria: 1) AST ≥ 20 U/L and ALT ≥ 40 U/L in males or ≥ 28 U/L in females 2) Waist circumference ≥ 85 cm in males or ≥ 90 cm in females 3) Diagnosis of metabolic syndrome having 2 or more of the following 3 risk factors at screening: a): Fasting plasma glucose ≥ 110 mg/dL or undergoing drug treatment for elevated glucose b): SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg or undergoing drug treatment for hypertension, or antihypertensive drug treatment in a patient with a history of hypertension	Liver stiffness measured by MRE >6.7 kPa

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	c): TGs ≥ 150 mg/dL or undergoing drug treatment for elevated TGss, and/or HDL-C < 40 mg/dL or undergoing drug treatment for reduced HDL-C	
4	Screening MRI -PDFF with ≥8% liver steatosis	Any of the following laboratory abnormalities: (1) ALT >5 × ULN or AST >5 × ULN (2) PT-INR ≥1.3 unless on anticoagulant therapy (3) Total bilirubin > ULN, except with an established diagnosis of Gilbert's syndrome (4) Platelet count < 80,000/μL (5) eGFR <45 as calculated by the BSA adjustment (normalized eGFR)

5	Fasting serum LDL-C > 120 mg/dL or receiving antidyslipidemic drugs	Acute or chronic liver disease other than NAFLD/NASH including but not limited to the following: (1) Hepatitis B (as defined by the presence of HB antigen at screening) or hepatitis C (as defined by the presence of HCV antibody [anti-HCV]) Patients with positive anti-HCV who test negative for HCV ribonucleic acid (HCV-RNA) at screening will be allowed to participate in the study as long as there is evidence of viral negativity for a minimum of 12 months before screening (2) Evidence of AIH (3) History of PBC, PSC, Wilson's disease, alpha-1-anti-trypsin deficiency, hemochromatosis or iron overload, drug-induced ALD, or known bile duct obstruction (4) Suspected or proven HCC
6	Be willing to maintain a stable diet and physical activity throughout the course of the study	Known history of HIV
7		Medical history of liver cirrhosis
8		Clinical evidence of portal hypertension to include any history of ascites, hepatic encephalopathy, or presence of esophageal varices
9		Use of drugs historically associated with NAFLD (amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, or valproic acid) or other known hepatotoxins for ≥2 weeks in the year before Screening

10		Use of the following medications: (1) GLP-1 agonists unless on a stable dose 3 months before screening or liver biopsy (2) Ursodeoxycholic acid or thiazolidinediones within 3 months before screening (3) Antidyslipidemic drugs have been stable for ≥3 months before Screening (4) Oral antidiabetic drugs have been stable for ≥3 months before screening (5) Agents (including herbal over-the-counter weight loss preparations) or medications known to significantly affect body weight within 3 months before screening
11		History of significant alcohol consumption, defined as an average of ≥20 g/day in female patients and ≥30 g/day in male patients, for a period of >3 consecutive months within 1 year before screening, hazardous alcohol use (Alcohol Use Disorders Identification Test score ≥8), or an inability to reliably quantify alcohol consumption but determined as alcohol polydipsia based on the judgment of the investigator or sub-investigator
12		Weight change of ≥10% within the 6 months before screening or ≥5% within the 3 months before screening
13		Surgery planned during the study period or after bariatric surgery (e.g., gastroplasty and Roux-en-Y gastric bypass)
14		Type 1 diabetes by medical history
15		Uncontrolled type 2 diabetes defined as hemoglobin A1c (HbA1c) of >9.5% at screening (patients with HbA1c >9.5% may be rescreened) or requiring insulin dose adjustment of >10% within 2 months before screening
16		Clinical hyperthyroidism or hypothyroidism or screening hormone results pointing to thyroid dysfunction. Patients receiving dose-stable thyroid replacement therapy for ≥3 months before screening will be allowed to

		participate in this study as long as thyroid tests show that the patient is euthyroid and stable
17		History of any condition causing malabsorption such as chronic pancreatitis, extensive bowel/small intestine surgery, celiac disease, or bile flow obstruction
18		History of any condition associated with acute or chronic diarrhea such as IBD, functional diarrhea, IBS with predominant diarrhea, IBS with mixed bowel habits, or unclassified IBS
19		Uncontrolled hypertension (either treated or untreated) defined as SBP of >160 mmHg or a DBP of >100 mmHg at Screening
20		A history of New York Heart Association (NYHA) Class III or IV heart failure, or known left ventricular ejection fraction of <30%
21		A history of myocardial infarction, unstable angina, percutaneous coronary intervention, coronary artery bypass graft, or stroke or major surgery within 6 months prior before screening
22		Active substance abuse, within 1 year before screening
23		Participation in an investigational new drug trial in the 30 days before screening or within 5 half-lives of an investigational agent, whichever is longer
24		Complication with malignancy Patients with a history of malignancies that have been treated with a curative intent or completed chemotherapy may be eligible Patients under evaluation for malignancy are not eligible
25		Known intolerance to MRI or conditions contraindicated for MRI procedures
26		Any other condition which is considered to be inappropriate for the study by the Investigator or sub-investigator

AIH, autoimmune hepatitis; ALD, alcoholic liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BSA, body surface area; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide-1; HBs, hepatitis B surface; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDL-C, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; LDL-C, low-density lipoprotein cholesterol; MRE, magnetic resonance elastography; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; PDFF, proton density fat fraction; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; PT-INR, prothrombin time-international normalized ratio; SBP, systolic blood pressure. TG, triglyceride, ULN, upper limit normal.

Randomization and masking

The patients will be randomized to each group (EXB+CTM, EXB, CTM, and PBO) at a ratio of 1:1:1:1 using a computer-generated centrally administered procedure (no factor for stratification). The contract research organization will create the list of study drug randomization and link the appropriate study drug number. After the investigators confirm the eligibility of participants, the required information will be entered in the EDC system, and the drug number will be issued. Investigators and patients will be blinded to the details of the assignment to conceal the drug allocation number in independent contact research organization until the key open. All trial drugs will be packed identically and identified only by the number assigned. As noted above, the treatment assignments will be fully masked from the patients and physicians.

Keycode break

If the investigator or sub-investigator considers it urgently necessary to break the study keycode prematurely, they will contact the person responsible for study drug randomization to file the request. This may occur due to a serious AE (SAE), the need for treating an AE, or a similar situation.

Harms and AE monitoring

AEs are defined as any unfavorable or unintended sign (including laboratory parameters and abnormal vital signs), symptom, or disease that may occur during the study period. AEs that are not directly related to the study drugs may develop. The investigator or sub-investigator will assess the severity of the AEs. Any AE that meets any of the following criteria will be considered an SAE: death, life-threatening, hospitalization requirement or prolonged hospitalization for treatment, disability, disability threat, other serious conditions, congenital disease, or anomaly in offspring. If

an SAE occurs, the investigator or sub-investigator will appropriately treat it, and the investigator will immediately report the details to the Hospital Director and the study drug supplier.

Study procedures

The investigator or sub-investigator will perform all observations, tests, investigations, and evaluations according to the descriptions provided in Table 3. If a blood test is scheduled at a study visit, blood will be collected only after the patient had fasted for at least 8 h. After the start of treatment, drug returns and blood test results will be checked to monitor adherence in each visit. Blood/stool sampling will be collected and stored for the exploratory analysis of genes (single Nucleotide Polymorphisms; patatin-like phospholipase domain containing 3 and Transmembrane 6 Superfamily Member 2), fibrosis, and inflammation etc., upon obtaining consent separately. When the study drug is given to the participants at each visit, the pharmacist will provide instructions on the dosage and administration. At the next visit, the pharmacist will ask the participants to bring the unused study drug in their next visit and record the number of tablets (packages) that are returned. These strategies will improve adherence to the intervention protocol.

Table 3. Schedule of observations, tests, and assessments

	Before Screening	Screening	Treatment Period				Follow-Up
		V1	V2/randomization	V3	V4	V5	V6/EOT	V7
Study week		Week -8 to day -1	Day -1	Week 4	Week 8	Week 12	Week 16	EOT to 2 weeks
Visit window			-	±7 days	±7 days	±7 days	±7 days	±7 days
Informed consent	○							
Inclusion/exclusion criteria		○	○					
Demographics		○						
Serology [a]		○						
Chest X-ray		○						
ECG		○						
Physical examination [b]		○	○				○	
Vital signs [c]		○	○	○	○	○	○	○
Pregnancy test [d]		○	○				○	
MRI [e]		○					○	

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Liver biopsy		△						
Randomization			○					
Hematology/urinalysis [f]		○	○[k]	○	○	○	○	○
Endocrinology		○						
Biochemistry 2 [g]		○	○[k]				○	
Total bile acids			○[k]				○	
Lipid profile		○	○[k]	○	○	○	○	○
Others [h]			○[k]				○	
Blood/stool sampling for storage			●				●	
Dispense study drug			○	○	○	○		
Review study drug compliance				○	○	○	○	
Review alcohol consumption [i]		○						
Review concomitant medications		○	○	○	○	○	○	
Review adverse events		○	○	○	○	○	○	○
Questionnaire/review patient diary [j]			○	○	○	○	○	

○: To be performed

△: Information will be collected from patients who have liver biopsy results.

●: Blood/stool sampling for storage will be collected for the analysis of genes, fibrosis, and inflammation etc, upon obtaining consent separately.

[a]: Includes HB antigen, HCV antibody, and HCV-RNA.

[b]: Includes height (at V1 only), body weight, waist circumference, and waist-to-hip ratio. BMI will be calculated based on height and weight.

[c]: Vital signs include blood pressure, heart rate, respiratory rate, and axillary temperature.

[d]: For women of childbearing potential, a urinary pregnancy test will be performed at V1, V2, and V6. The test is not required at V2 when it occurs within 1 month after V1.

[e]: Patients will undergo an MRI to measure liver fat (PDFF) and total liver volume. Patients who discontinue before V6 (week 16) should undergo an MRI at the end of treatment if they completed at least 4 weeks of treatment.

[f]: Hematology/urinalysis includes hematology/coagulation, biochemistry 1, and urinalysis.
[g]: Biochemistry 2 includes glucose, HbA1c, and insulin.
[h]: Others include high-sensitivity CRP, type IV collagen 7s, FIB-4, and APRI.
[i]: History of alcohol consumption will be obtained at screening.
[j]: Questionnaires of PA-QOL and CLDQ will be answered at each visit. Number of bowel movements and BS score will be collected from the patient diary.
[k]: Data within 1 month can be used as a substitute.

Concomitant treatment

The administration of the following medications and therapy are prohibited from the time of Screening to the end of follow-up: bile acid agents (ursodeoxycholic acid, chenodeoxycholic acid, and dehydrocholic acid), ileal bile acid transporter inhibitors, anion-exchange resin agents other than the study drugs, thiazolidinedione, and bariatric surgery. The restricted concomitant medications are permitted only if they are continuously used at a stable dose within 4 weeks of the study enrollment. The dose and dosage regimen of the following drugs must be determined to be within the stable range at the start of study treatment and kept stable until the end of study treatment: antihypertensive drugs (angiotensin II receptor blockers only); Vitamin E; antidyslipidemic drugs; and antidiabetic drugs (DPP-4 inhibitors, GLP-1 receptor agonists, and insulin injections).

Criteria and procedure for withdrawal from the study

The investigator or sub-investigator will discontinue the study enrollment of a patient if the participant meets any of the following criteria: (1) the patient desires withdrawal, (2) after enrollment, the patient is found not to meet the inclusion criteria or exclusion criteria, (3) if the investigator or sub-investigator opines that having the patient continue in the study is not appropriate due to an AE, and (4) if the investigator or sub-investigator opines that having the patient continue in the study is not appropriate due to any other reason.

Efficacy evaluation

The primary efficacy endpoint will be the absolute change in LDL-C level from the baseline to 16 weeks after treatment initiation. The exploratory endpoints are provided in Table 1. The assessment of the MRI-PDFF/MRE will be performed by an independent liver specialist who is blinded to the treatment (IK).

Safety assessments

The following safety evaluations will be performed during each patient visit from the time of treatment initiation until the 2-week follow-up period: (1) incidence of AEs in the treatment groups

compared with that of the placebo groups, (2) change in the daily score of the Bristol Stool Form Scale from the baseline regarding the number of bowel movements and the Patient Assessment of Constipation Quality of Life (PAC-QOL), (3) change in the Chronic Liver Disease Questionnaire (CLDQ) scale from the baseline.

Population analysis

The set of subjects to be analyzed will be determined before locking the data of each patient and will be defined as follows. The modified intention-to-treat, which is the full analysis set (FAS), and per-protocol set (PPS) will be used for the assessment of primary efficacy. The FAS will include all patients who are randomized, except those who meet any of the following criteria: (1) patients with major protocol deviation (e.g., deviation of informed consent and major deviation in the study procedures), (2) patients who have not received any dose of the study drugs, and (3) patients who have no measurement of the efficacy endpoint. A PPS will include patients without protocol deviations. The Safety Analysis Set (SAS) will be used for safety assessment and will include all patients who receive at least one dose of the study drug.

Statistical analysis

Considering the placebo-controlled randomized parallel-group comparison design, we will use descriptive statistics to summarize differences between the placebo group and each monotherapy or combination group and those between each monotherapy group and the combination group. Unless otherwise noted, the descriptive statistics include the number of patients, mean, standard deviation, median, minimum and maximum values of continuous variables, frequency, and percentage of categorical variables.

For the primary endpoint, the change in serum LDL-C from the baseline to week 16 (difference in measurement values between time points) will be summarized for each treatment group. Point estimates and confidence intervals for the mean difference from the baseline between the placebo group and each monotherapy or combination group and those between each monotherapy group and the combination group will be calculated. The changes over time in the measured parameters will be summarized for each treatment group. The ratio of change (ratio of the absolute change to the baseline value) will also be analyzed using the same methods. The exploratory endpoints will be analyzed similar to the primary endpoint. Due to the exploratory nature of this study, a concrete statistical hypothesis is not formulated. For exploratory purposes, in case any statistical test is performed or confidence interval of any parameter is calculated, the significance level of 5% (two-sided) and the confidence coefficient of 95% will be used. Multiplicity arising from the interpretation of statistical tests or confidence intervals will not be adjusted.

Interim analysis

Not applicable.

Data management, central monitoring, and audit

The investigators' sites will maintain individual records of each patient as source data, which include a copy of informed consent, medical records, laboratory data, and other records or notes. All data will be collected by the independent data management center. The data management center will oversee the interstudy data sharing process. The clinical data entry, data management, and central monitoring will be performed using the electric data capture VIEDOC 4 (PCG Solutions, Sweden). Furthermore, auditing will be planned at an external clinical research organization.

Efficacy and safety assessment board

Although no interim analysis will be performed in this study, this committee was established to monitor the overall study focusing on safety, as this is the first study to administer elobixibat and cholestyramine in combination to patients with NAFLD. The board will consist of three external board members who have no relationship with the operations involved in the study.

Study flow and schedule of enrolment, interventions, and assessments

A study flowchart is shown in Fig. 1. The study schedule is presented in Table 3.

Patient and public involvement

In this randomized controlled trial, patients will be involved in the recruitment to and conduct of the study. Especially, the development of the research question and outcome measures were based on patients' priorities, experience, and preferences. The results of this study will be disseminated by email to the participants who indicate that they want the results. The burden of intervention will be assessed by patients before the commencement of the trial; patients' satisfaction of the treatment will be collected as a part of the post-intervention assessment.

Ethics and dissemination

This study will be conducted in compliance with the Declaration of Helsinki, "Order for Enforcement of the Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices," and Good Clinical Practice (GCP) standards. The study protocol and relevant supporting data were approved on November 26, 2019 by the institutional

ethics committee before participant enrollment. The trial results will be reported according to the Consolidated Standards of Reporting Trials 2010 guidelines. This trial has been registered with ClinicalTrials.gov registry (NCT04235205) and will be overseen by an external monitor and clinical research organization. Written informed consent (see supplementary file for informed consent form) for study participation will be obtained from all enrolled participants. The amended or modified study protocol will be approved by the IRB. The results of this study will be submitted for publication in international peer-reviewed journals, and the key findings will be presented at conferences. The funder has no role in the study design, data collection, or data analysis. Participants will be informed of the results of the trial by the investigators. Authorship will be ascribed in accordance with the International Committee of Medical Journal Editors guidance.

DISCUSSION

This is the first proof-of-concept study proposed to explore the effect of EXB in combination with CTM in patients with NAFLD. A previous study showed that an IBATi improved both hepatic and whole-body aspects in a mouse model of NAFLD.[9] However, EXB causes AEs, including abdominal pain and diarrhea, by increasing colonic BAs, resulting in the development of BA-induced diarrhea. Therefore, the safety and tolerability of monotherapy with EXB might decrease in patients with NAFLD. Considering the MOA, the removal of excess BAs from the colon using CTM has the potential to improve BA-induced diarrhea.

In some well-known trials (e.g., PIVENS, FLINT, and GOLDEN),[15, 18-20] the primary endpoints included liver histology, which was evaluated using liver biopsy specimens. Liver histology endpoints, such as the complete resolution of NASH, are considered surrogates for preventing cirrhosis because they are considered predictive of clinical benefit but are not direct measures. Currently, the only method to diagnose NASH is a liver biopsy, as described in the guidelines (“gold standard”). However, in Japan, the number of patients with NAFLD exceeds 10 million, and it is difficult to perform liver biopsy for all NAFLD patients. In addition, liver biopsy has various problems such as invasiveness, cost, and differences in interpretation among pathologists.

In recent years, the progress of MRI technology has been remarkable, and it has become possible to accurately diagnose steatosis and fibrosis using MRE.[21, 22] Quantification of body fat percentage using MRI is also possible by using echo asymmetry and least-squares estimation sequence methods to measure PDFF by repeated decomposition of water and fat.[17, 23] MRE and MRI-PDFF can be performed at the same time in one imaging, and the combined results can evaluate liver steatosis and liver fibrosis. Non-invasive evaluation of liver fibrosis and liver steatosis using MRI has the potential to replace liver biopsy in the clinical setting.[17] Hence, we chose MRI for the noninvasive assessment

of hepatic steatosis in this study. Quantification of liver fat content using MRI-PDFF has been previously shown to be sensitive in detecting changes, and it has been used in nonalcoholic steatohepatitis clinical trials for quantitative fat assessment.[17, 21]

Our study has the following strengths: (1) the first randomized, placebo-controlled, double-blinded study focused on removing BAs in patients with NAFLD; (2) a novel POC study considering the drug MOA for improving the treatment effect and tolerability, as well as reducing the AEs; (3) MR images will be captured following a standardized protocol and processed under the supervision of a hepato-radiologist blinded to the study; (4) the comparison of colocalized regions of interest for fat content changes in each of the nine liver segments between weeks 0 and 16; (5) the final assessment of the MRI examinations will be randomized with regards to time points in order to reduce bias; and (6) the measurement of exploratory endpoints such as the lipid class, endocrine functions, BA, inflammation, fibrosis, gut-microbiota, and trimethylamine-N-oxide (Table 2). Nevertheless, our study also has the following limitations: (1) will be performed in a single center; (2) a relatively small sample size; (3) a relatively short treatment duration; and (4) the lack of liver biopsy.

NAFLD/NASH is a heterogeneous disease with correspondingly complex pathophysiology, which includes redundant pathways that may not be uniform among patients. Considering the MOA of pharmaceuticals for treating NAFLD/NASH, its complex pathophysiology permits the development of a wide array of potentially viable therapeutic targets, especially components of anti-metabolic, anti-inflammatory, and anti-fibrotic pathways.[24] In a phase IIa trial for NASH/NAFLD therapies, BMS-986036 (FGF21) [25] and NGM282 (FGF19) [26] exerted an anti-metabolic effect, and the liver fat content was used as the primary endpoint. Although the current NAFLD therapy focuses on anti-metabolic, anti-inflammatory, and anti-fibrotic aspects in the liver as MOA, decreasing BAs for treating NAFLD is a novel therapeutic target for patients with NAFLD. EXB–CTM combination therapy-associated improvements in the changes of the absolute and relative liver fat levels changes are clinically relevant.

DECLARATIONS

Ethics approval

The Ethics Committee of Yokohama City University Hospital approved the study protocol on November 26, 2019. This trial is registered with ClinicalTrials.gov (number NCT04235205).

Confidentially

Data will be retained in accordance with the Japanese ethical guidelines for clinical research. Participants will be allocated a unique identification (ID) number at entry. The master list linking participant personal information and ID number will be maintained in a separate locked cabinet and

password-protected hard drive. Data will be analyzed by ID number only. Records will be retained for 5 years after study completion, and then destroyed by the data center.

Competing interests

AN reports grants and research support from Gilead, Mylan EPD, EA Pharma, Kowa, Taisho, Biofermin; is a consulting adviser for Gilead, Boehringer Ingelheim, BMS, Kowa, Astellas, EA Pharma, Mylan EPD. Other authors declare no competing interests.

Funding

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Authors' contributions

TKessoku and AN participated in study design. TKessoku and TKobayashi conducted feasibility phase work. Recruitment of participants and follow-up will be performed by TKessoku, AO, MI, TKobayashi, YH, YO, MY, and SS. Reading of MRI will be done by KI. Exploratory item will be measured by HU and KW. TKessoku, TKobayashi, YS, KY, TY, and AN will participate in data interpretation. All authors contributed to writing, and all read and approved the final manuscript.

Acknowledgments

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Data sharing statement

Data are available upon reasonable request.

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FIGURE LEGEND

Figure 1. Study design. [a]N = 100 enrolled. CTM, cholestyramine; EXB, elobixibat; NAFLD, nonalcoholic fatty liver disease; PBO, placebo; QD, quaque die.

Figure 1

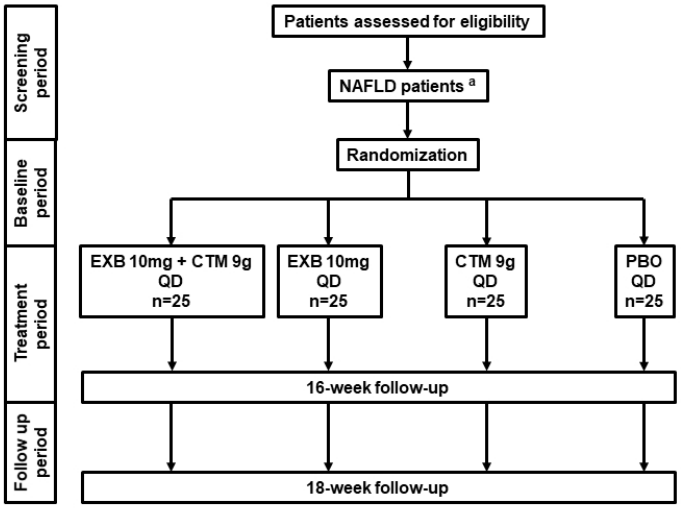

Figure 1

254x190mm (96 x 96 DPI)

Information Document / Consent Form

For Patients

Title of Clinical Trial: A Phase II Investigator-led Study to Compare the Efficacy and Safety of Combined Elobixibat and Cholestyramine to that of Placebo, Cholestyramine Monotherapy or Elobixibat Monotherapy for Nonalcoholic Fatty Liver Disease

Contents

1. Introduction

We would now like to provide you with explanations about the clinical trial on the candidate drugs AJG533 (Elobixibat) and CTM27 (Cholestyramine). Please decide whether or not you would like to participate in this study, after listening to the explanations carefully and understanding what you are being told. You may take this written information document home and discuss the participation with your family. You are not being compelled to participate. Whether or not you participate in the study is entirely up to you. Even if you agree to participate in the study, you may stop doing so at any time without giving a reason. Furthermore, please understand that you will not be mistreated or lose any healthcare benefits for refusing to participate or withdrawing your consent to participate during the study.

If you would like more explanations or have questions you wish to ask, please do not hesitate to ask the investigator.

2. What is a Clinical Trial?

In order to have patients use a new drug, the Ministry of Health, Labour and Welfare must have checked the drug for its medicinal effect (efficacy) and for its undesirable effects (side effects). The process of drug development begins with a search for a “Drug candidate” as shown in Fig. 1-1 (Step 1). This substance is then tested in animals to see what kind of an effect it has (Step 2). The safety of the substance as a drug is then checked by having healthy volunteers use it, and patients are recruited to investigate the “efficacy” and “safety” of the drug (Step 3). A study that investigates a drug in this manner is called a “Clinical Trial”. A drug candidate that is investigated in such a study is called a “Study Drug”. Clinical trials are conducted based on the “Good Clinical

Practice (GCP) Standards" outlined by the government, with respect to the human rights and safety of its participants.

The results of clinical trials conducted with the help and cooperation of many patients are then compiled so that the government (MHLW) may ultimately review the data to see whether the substance can be approved as a "Drug" (Step 4). Once approved, it is ready for common use (Step 5). All drugs that we use today have had their efficacy and safety checked through such clinical trials.

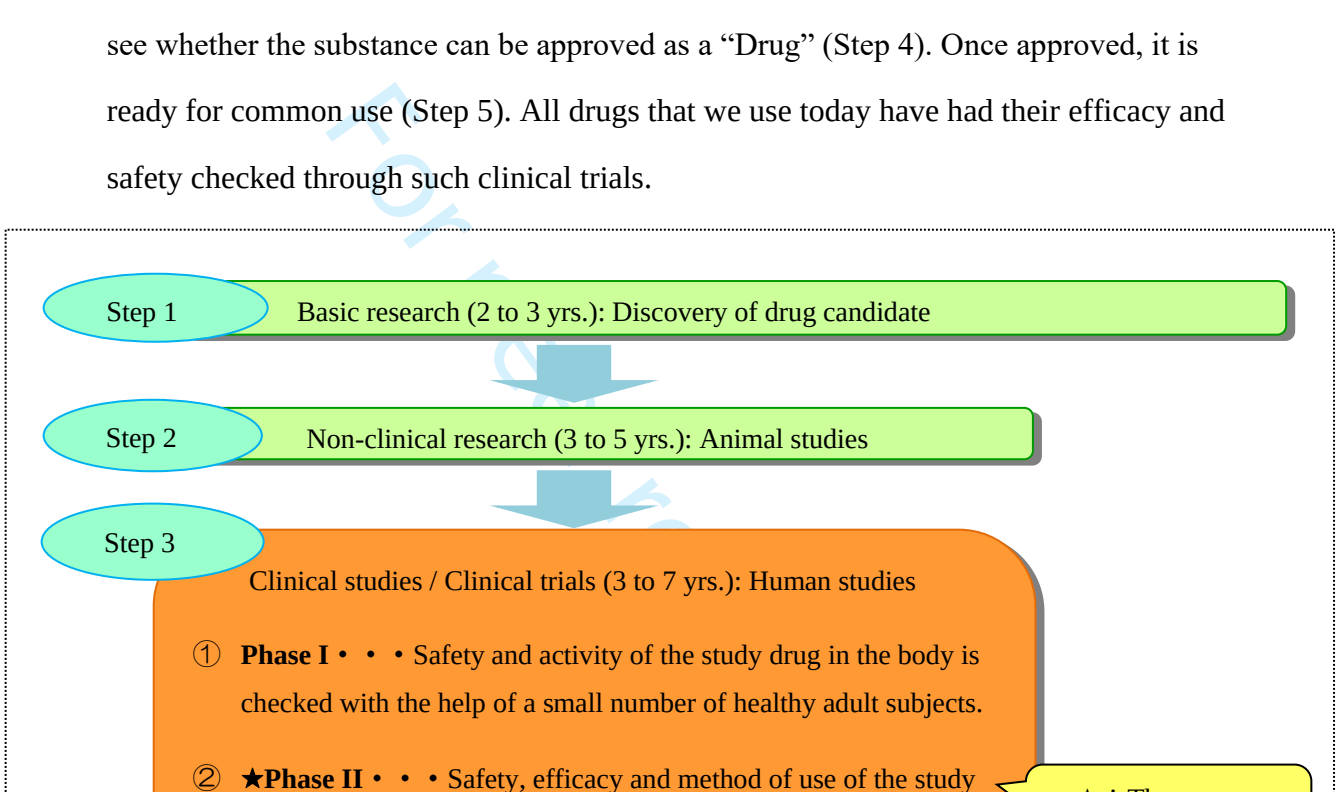

Figure 1-1 Flow of events leading to the birth of a new drug

In addition to the purpose of gaining necessary information for reviewing the drug, clinical trials also contain research aspects to it to gain new and unseen information. For this reason, clinical trials are conducted in accordance with the strict rules and standards stipulated by the government, and by thorough review and approval by an Institutional Review Board to ensure the scientific and ethical validity of the study plan, as well as its problem-free implementation. Please note that participation in a clinical trial will require you to make more hospital visits and undergo more tests than routine medical care.

Institutional Review Board (IRB)

This hospital has a clinical research review committee, or Institutional Review Board (IRB), which has been established by the director of the hospital and consist of physicians, other hospital staff and members of the general public that are outside of the hospital. This study has been reviewed and approved by this IRB to ensure that there are no scientific or ethical problems associated with it, and that the physician(s) involved with this study are eligible to do so. In addition, the IRB will examine the validity of continuing the study.

You can access the procedure manual and contents of review of the IRB on the website indicated below, and at the Office of Clinical Research Management. For details, please do not hesitate to contact the investigator or the Clinical Trial Help Desk.

＜Institutional Review Board of this hospital＞

Established by: Director of the Yokohama City University Hospital
Name: Yokohama City University Hospital IRB
Format: IRB established in the study site
Location: 3-9 Fukuura, Kanazawa-ku, Yokohama-shi, Kanagawa Prefecture
Website URL: http://www-user.yokohama-cu.ac.jp/~ynext/trial/irb/

3. About Your Disease (Symptoms)

The disease you have is called nonalcoholic fatty liver disease, where fat accumulates in the liver despite you not drinking much alcohol. It is thought to be caused by overeating, obesity and lack of exercise (a form of disease that is not caused by excessive consumption of alcohol). The disease can be classified into two types, the first being the “nonalcoholic fatty liver disease” that advances slowly, and the “nonalcoholic steatohepatitis” that advances rapidly and involves inflammation in addition to nonalcoholic fatty liver disease. In some cases, deterioration of the fatty liver disease can lead to liver cirrhosis and liver cancer.

4. About the Study Drugs “AJG533 (Elobixibat)” and “CTM27 (Cholestyramine)”

One of the study drugs that you will take upon agreeing to participate in this study, AJG533 (Elobixibat), has been approved in 2018 to be used to treat “Chronic constipation”. The other study drug, CTM27 (Cholestyramine), has been approved in 1984 to treat “Hypercholesterolemia”. These study drugs are expected to improve nonalcoholic fatty liver disease by preventing the absorption of bile acids by the body and quickening fat processing by the liver.

The present study will use drugs that contain the active ingredients of Elobixibat and Cholestyramine, as well as drugs that resemble but do not contain the active ingredients of Elobixibat and Cholestyramine (placebo drugs) as the study drugs.

5. Purpose of the Study

The purpose of this clinical trial is to investigate the efficacy and safety of the study

drugs by having patients of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis take the study drugs once a day. To be specific, we will make comparisons between patients that take either Elobixibat or Cholestyramine alone to patients that take placebo (drugs that resemble the actual drugs but contain no active ingredients), as well as comparisons between patients that take both Elobixibat and Cholestyramine to patients that take either Elobixibat or Cholestyramine alone. Please refer to "6. Study Methods (3) Method of use of study drugs" for details on how the drugs will be taken.

This hospital is the only site in which this clinical trial is being conducted, and we expect to recruit around 100 patients to participate in it.

The Elobixibat to be used in this study will be provided based on an appropriate agreement by EA Pharma Co., Ltd., who is selling a drug that has the same active ingredient. Furthermore, this clinical trial is being conducted with financial assistance from EA Pharma Co., Ltd. However, there will not be any changes made to your treatment or actions taken to compromise the impartiality of this study to favor the profits of EA Pharma Co., Ltd.

6. Study Methods

Criteria to participate in the clinical trial

(1) Clinical trial participation criteria

In order to carry out this study safely, those who participate must have fulfilled the conditions indicated below. Potential participants will be asked to undergo additional tests, and the investigator will ultimately decide if a patient can participate in the study. A patient may not be able to participate in the study depending on the outcome of these tests.

1) Individuals that can participate in the study

① A patient that is 20 to 75 years old at the time of consent

② A patient who can give his/her own written consent

③ A patient with definitive diagnosis of nonalcoholic steatohepatitis or suspected of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis based on a liver biopsy (a test takes a piece of liver tissue to diagnose the disease) within 8 months prior to the start of the screening period of this study

④ A patient whose liver fat mass as seen by MRI scans meets certain criteria

⑤ A patient whose fasting serum LDL cholesterol level meets certain criteria, or is receiving drug treatments for dyslipidemia

⑥ A patient capable of maintaining a stable diet and routine life during study participation

⑦ A patient deemed eligible by the investigator

2) Individuals that may be refused entry to study

① A patient that is pregnant, breastfeeding or may be pregnant at present time, or cannot agree to practice contraceptive methods during study participation

② A patient whose MR elastography results, BMI, hematological findings (liver function tests, platelets etc.) and urinalysis results fail to meet certain criteria

③ A patient with history of or complications with an acute or chronic liver disease that is not nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (active

viral hepatitis, cholangitis, bile duct obstruction, suspicion of hepatocellular carcinoma, Wilson disease, α1-antitrypsin deficiency, hemochromatosis or iron overload)

④ A patient with history of liver cirrhosis, HIV infection, type 1 diabetes, chronic pancreatitis, extensive colon/small intestinal surgery, Celiac disease

⑤ A patient with complications due to portal hypertension (ascites, hepatic encephalopathy, esophageal varices)

⑥ A patient that used drugs for treating nonalcoholic fatty liver disease for a period of 2 weeks or more during the one year leading up to the start of the study

⑦ A patient who has used ursodeoxycholic acid, thiazolidinedione, or any of the drugs known to have a significant effect on body weight before the start of the study

⑧ A patient that has used dyslipidemia medicines or oral diabetes medicines before the start of the study and has changed the doses used for them

⑨ A patient that fails to meet certain criteria regarding history of alcohol consumption

⑩ A patient who fails to meet certain criteria regarding the changes in body weight prior to the start of the study

⑪ A patient that has undergone surgery for obesity, or plans to do so during study participation

⑫ A patient with type 2 diabetes that his/her physician deems difficult to control

⑬ A patient with complications of hyperthyroidism or hypothyroidism, or thyroid

dysfunction, and fails to meet certain criteria

⑭ A patient with history of an illness associated with acute or chronic diarrhea

⑮ A patient whose blood pressure does not meet certain criteria

⑯ A patient who fails to meet certain criteria due to abnormalities of the heart or history of cerebral strokes

⑰ A patient with history of drug abuse during the one year leading up to the start of the study

⑱ A patient who has participated in another clinical trial during a certain period prior to the start of the study

⑲ A patient who has complications with malignant tumors

⑳ A patient who is unable to undergo MRI scans

㉑ A patient deemed ineligible by the investigator for other reasons

(2) Duration of study participation

Your participation in this study is scheduled to last a total of 26 weeks (up to around 6 months), which includes up to 8 weeks of Screening Period, 16 weeks of Study Drug Treatment Period and 2 weeks of Follow-up Period. Even if you discontinue your participation during the Study Drug Treatment Period, you will be asked to participate in the Follow-up Period (2 weeks after discontinuation).

(3) Method of use of study drugs

Once patients are confirmed to meet the "(1) Clinical trial participation criteria" prior to the start of the study, they are assigned to one of the following treatment groups and receive study drug treatments for 16 weeks. You will be randomly assigned to a group according to a predetermined selection method, and neither you nor the investigator will be able to choose the group to which you end up in. Furthermore, as study drugs are made to be indistinguishable from one another, you will know neither the identity of the drug nor the group to which you are assigned. This is to ensure the proper evaluation of the efficacy and safety of study drugs without the influence of preconception.

[Treatment Groups] There are 4 treatment groups.

Group 1	Elobixibat (Placebo) 2 tablets + Cholestyramine (Placebo) 2 sachets
Group 2	**Elobixibat 2 tablets** + Cholestyramine (Placebo) 2 sachets
Group 3	Elobixibat (Placebo) 2 tablets + **Cholestyramine 2 sachets**
Group 4	**Elobixibat 2 tablets + Cholestyramine 2 sachets**

[How to take the study drugs]

• You will be asked to come to the hospital 1 day before the start of treatments. You will start taking the study drugs from before breakfast on the following day, and continue to do so until before eating breakfast on the visit date 16 weeks later.

• You will be asked to take the study drug once a day, before breakfast. You will take 2 tablets of Elobixibat (including placebo) and 2 sachets of Cholestyramine (including placebo) each time.

• If you do not manage to take the study drugs at its intended timing, please take it within 12 hours or before 21:00, whichever is earlier.

• Please store the study drugs away from areas of high temperature and humidity.

(4) Schedule

After you agree to participate in this study, you will undergo tests and observations described in Table 6-1 according to schedule. In addition, you may be required to make additional visits, undergo additional tests and have additional blood samples taken aside from those which have been scheduled based on changes in your condition and by instruction of the investigator. The specific schedule is as outlined below.

1) Screening Period

If you provide your consent to participate in this study, you will be asked to undergo tests so that we may check that your present condition meets the criteria for you to participate in the study.

2) Treatment Period

Patients deemed eligible to participate in this study according to screening tests will be asked to take the study drugs. During the Study Drug Treatment Period, patients will undergo prescribed tests, and have their condition and clinical course of the disease checked to determine whether or not it is appropriate for them to continue in the study.

3) Follow-up Period

You will also be asked to undergo medical examinations and tests to check your condition 2 weeks after the end of study drug treatments.

4) Discontinuation

If you discontinue study drug treatments prematurely during the Study Drug Treatment Period, you will be asked to undergo medical examinations and tests when you stop taking the study drugs. In principle, you will also be asked to come to the hospital 2 weeks after discontinuation of treatment to undergo medical examinations and tests.

Table 6-1. Study Schedule

Item	Before screening	Screening	Treatment					Follow-up
Weeks		-8 weeks to day before start of treatment	1 day before start of treatment	Week 4	Week 8	Week 12	Week 16/Discontinuation	End of treatment to 2 weeks
Tolerable range				±7 days	±7 days	±7 days	±7 days	±7 days
Consent	●							
Investigation of alcohol consumption history		●						
Chest X-ray		●						
ECG		●						
Height[1)]/Body weight/Waist/hip circumference/Waist/hip ratio		●	●				●	
Blood pressure/heart rate/respiratory rate/axillary body temp.		●	●	●	●	●	●	●
Pregnancy tests[2)]		●	●*				●	
MRI scans		●					●	
Liver biopsy[3)]		△						
Blood tests[4)]/Urinalysis		●	●*	●	●	●	●	●
Blood/fecal sampling for storage[5)]			○				○	
Questionnaire/Patient diary check			●	●	●	●	●	
Concomitant drugs		←	—	—	—	—	—	→
Adverse events		←	—	—	—	—	—	→

1) The height is only measured during screening.

2) Only the relevant female patients will undergo urinary pregnancy tests at screening, 1 day before start of treatment, and at Week 16 / Discontinuation.

3) Information will be collected for those who have results of a prior liver biopsy.

4) Blood sampling: Up to around 20 mL of blood will be taken each time, and up to around 100 mL of blood will be taken in total over the course of the study.

5) Blood and fecal samples for storage will be collected if you consent to providing them for exploratory research planned for the future. Up to around 20 mL of blood will

be collected. The blood and fecal samples collected will be used in exploratory research planned separately from this study, and approved by an ethics review committee that deliberates the implementation of said study from an independent and impartial standpoint. In this situation, your samples will be used to analyze inflammation after asking for your consent again. These samples will be stored for up to 5 years from the moment of their collection, and will be discarded according to appropriate methods thereafter.

*If there is data from less than one month ago, this will be used instead of conducting a new test.

7. Expected Benefits and Side Effects

＊Expected benefits

By using Elobixibat and Cholestyramine to suppress the absorption of bile acids by the body and quickening fat processing by the liver, we can expect to see improvements in nonalcoholic fatty liver disease.

＊Expected disadvantages

Although this study will assign each patient to a group to receive placebo, Elobixibat, Cholestyramine or combined Elobixibat + Cholestyramine, not all patients of all groups are guaranteed to see any therapeutic benefit. We do not expect patients that are assigned to the “Placebo group” to receive any therapeutic benefits of a drug.

In addition, clinical trials tend to involve more examinations and tests, and the examinations tend to take a lot of time. There are also drugs and treatments that you

cannot receive while you are participating in the study. Please notify the investigator in advance if you will receive any of the following drugs or treatments during your participation in this study.

[Drugs you cannot use during the study]

Drugs you cannot use during the study	Period
Bile acid preparations (ursodeoxycholic acid, chenodeoxycholic acid, dehydrocholic acid)	From screening to end of follow-up
Bile acid transporter inhibitors (Elobixibat) and anion exchange resin preparations (Cholestyramine, Colestimide) other than study drugs	
Thiazolidinedione	

[Treatments prohibited during the study]

Type of treatment	Period
Obesity surgery (sleeve gastrectomy, gastric bypass, sleeve bypass etc.)	From screening to end of follow-up

[Drugs that should be co-administered with great care during the study]

Type of drug	Period
Cardiotonic drugs (Digoxin, dabigatran etexilate methanesulfonate)	From the start to the end of study drug treatments
Aluminum-containing preparations	
Midazolam	
Anti-rheumatic drugs (methotrexate, salazosulfapyridine)	
Non-steroidal anti-inflammatory drugs	
Corticosteroids (hydrocortisone)	
Immunosuppressant drugs (Mycophenolate mofetil)	
Thiazide antihypertensive diuretic	
Chlorthalidone	

Type of drug	Period
Meticrane	
Mefruside	
Tetracycline	
Phenobarbital	
Vancomycin hydrochloride	
Thyroid hormone preparations	
Raloxifene hydrochloride	
Fibrate drugs	
Warfarin	
Fluvastatin sodium etc.	
Ezetimibe	
Acarbose	
Spironolactone	

[Drugs whose method of use cannot be changed or whose use cannot be newly started during the study]

Type of drug	Period
Antihypertensive drugs (angiotensin II receptor antagonist only)	From 4 weeks before enrollment to the end of study drug treatments
Vitamin E	
Dyslipidemia medication	
Diabetes medication (DPP-4 inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, insulin injections)	

＊Side effects

Various studies have been conducted in Japan and abroad using Elobixibat and Cholestyramine, and the following side effects have been observed in these studies. It does not mean that all side effects would manifest in all patients. On the other hand, it

is possible that side effects other than those listed here would appear, and the possibility cannot be ruled out that some side effects can be life-threatening.

○Safety information about Elobixibat being marketed in Japan (product name: Goofice tablet 5 mg)

Elobixibat is a drug that is already being sold in Japan for the purposes of improving the symptoms of chronic constipation, and its use is associated with side effects described below. As such, similar side effects may appear.

In Japanese clinical studies conducted until drug approval, 292 out of 631 subjects (46.3%) experienced side effects of the drug. The main side effects that appeared were abdominal pain in 120 subjects (19.0%), and diarrhea in 99 subjects (15.7%).

Although there were no serious side effects, the other side effects observed were as shown below, according to their frequencies.

	5% or more	1 to 5%	Less than 1%
Liver		Liver function test abnormalities (increased ALT and AST)	
Psychological / Neurological			Headaches, dizziness
Circulatory			Hot flashes
Gastrointestinal	Abdominal pain (19.0%), diarrhea (15.7%), lower abdominal pain, abdominal distension	Nausea, upper abdominal pain, abdominal discomfort, soft stool	Bloating, dry mouth, defecation urgency, vomiting, abnormal intestinal sounds, constipation, stomatitis
Hypersensitivity			Hives, rash
Blood			Increased eosinophils, anemia, increased Vitamin E
Other		Increased CK	Dysmenorrhea

○Safety information about Cholestyramine being marketed in Japan (product name: Questran powder 44.4%)

Cholestyramine is a drug that is already being marketed in Japan for improving the symptoms of hypercholesterolemia in adults, and its use is associated with side effects described below. As such, similar side effects may appear.

In Japanese clinical studies and drug use surveys conducted until drug approval, 272 out of 1,594 subjects (17.1%) experienced side effects of the drug. The main side effects that appeared were constipation in 174 subjects (10.9%) and increased ALT in 85 out of 1,369 subjects (6.2%).

Intestinal obstruction (frequency unknown) was observed as a serious side effect, and the other side effects observed were as shown below, according to their frequencies.

	5% or more	0.1 to 5%	Less than 0.1%
Gastrointestinal	Constipation	Hard stool, gastric/abdominal distension, abdominal murmurs, loss of appetite, nausea and vomiting, diarrhea, soft stool, abdominal pain, epigastric pain, gastric/abdominal discomfort, heartburn	Gastric ulcers, gingival swelling
Liver	Increased ALT	Increased AST, increased Al-P, increased LDH, increased total bilirubin, abnormal liver function	
Kidneys		Increased BUN, increased creatinine	
Blood		Increased white blood cells, decreased hemoglobin, decreased hematocrit	
Skin		Rash, pruritus	Facial flush, heat sensation, erythema
Muscles		Increased CK	
Other		Oral aphtha, increased serum potassium/phosphorus/uric acid, decreased serum potassium/calcium/Vitamin D	Lightheadedness, headaches, vitreous hemorrhage, tinnitus, capillary expansion, urinary disorder, fatigue

Not all patients necessarily experience these side effects. Please notify the investigator immediately if you notice any changes to your condition or symptoms that bother you. You will receive appropriate treatments for it. Regular tests and examinations will be carried out during the study to check for the appearance of such undesirable symptoms. Unforeseeable side effects other than those listed here may appear. Please speak to the investigator at any time to find out about the latest information related to side effects.

8. When New Information about the Study Becomes Available

If any changes will be made to the plan of this study, you will receive explanations about what is being changed.

In addition, should we obtain new information that can affect your wish to participate in this study during the study, such as information about new side effects, we will notify you about it immediately. In these situations, we will ask you again if you wish to continue participating in the study.

However, as described in "9. Discontinuation of the Study", the decision to participate in this study is entirely yours. As such, you may withdraw yourself from the study at any time.

9. Discontinuation of the Study

Even if you start participating in this study, the study may be discontinued in the following situations:

(1) Discontinuation of the clinical trial itself

1) Due to unavoidable circumstances that affect the safety of patients, or the ethical or medical aspects of the study
2) Due to loss of scientific validity for developing this drug
3) Due to hindrance of proper conduct of the clinical trial resulting from violation of ministerial ordinances, study plans and various procedures that the Principal Investigator or study site should abide by
4) Due to decision by the Principal Investigator to terminate or suspend this study
5) Due to an instruction by the IRB to terminate the study

(2) Discontinuation of your participation in the study

1) Due to your request to withdraw from the study
2) Due to test results and your symptoms not conforming with the conditions for you to participate in the study
3) Due to your poor physical condition that makes it difficult for you to continue the study
4) Due to other reasons for which the investigator recommends the discontinuation of your participation

If your participation in the study will be discontinued after you start the use of study drugs, please cooperate with the tests indicated by ● in the "Week 16/Discontinuation" column on "Table 6-1. Study Schedule".

Furthermore, you will not be disadvantaged or mistreated in any way in receiving your subsequent treatments, even if you discontinue your participation in the study. The best available treatment will be chosen to treat your disease. Please note that results of the study associated with you may continue to be used even after your withdrawal from the study.

10. Alternative Treatments

At present time, there is no insurance coverage in Japan for drugs that treat nonalcoholic fatty liver disease, and common approach to treat the disease is by improvement of lifestyle habits through diet and exercise therapy. This may improve background conditions such as obesity, diabetes, dyslipidemia and hypertension etc., and lead to improvements in nonalcoholic fatty liver disease as well. Although drugs for hypertension, dyslipidemia or diabetes are used indirectly to treat this condition if improvements in lifestyle habits do not lead to sufficient improvements in the condition, it is not clear whether these drugs can be effective in the long run. You will be able to

discuss with your doctor to choose a treatment that suits you best, regardless of whether you decide not to participate in this study or discontinue your participation in the study prematurely.

11. Health Injuries

If you notice any unusual symptoms during your participation in the study, please contact the investigator right away. Appropriate intervention and treatments will be given to you.

If you experience any health injuries such as side effects etc. during the study or after the end of the study as a result of your participation in the study, the investigator will make arrangements for you to receive the best available treatment for it. You may also be able to receive compensation for the health injuries. However, if the health injury resulted from you ignoring the investigator's instructions, or due to your negligence or intentional doing, as well as if it is learned that the health injury is unrelated to the study, your compensation may be void or restricted.

Please also remember to store the medical bills issued by the medical institution safe to receive compensation.

Please see the attachment ("Compensation for Health Injuries") for details about compensation.

12. Reducing Financial Burden associated with Study Participation

You will not be charged anything for the study drugs that you will take during the

study. They will be provided to you free of charge. However, you will have to pay for the insured medical treatment costs related to the medical examinations (blood tests, MRI scans, etc.) performed at this hospital as well as drug charges other than the study drugs.

This hospital will pay you 10,000 JPY per visit as burden-relieving payments to reduce your financial burden, such as transportation costs. You may be paid up to 70,000 JPY in total, according to the number of study visits you complete. These payments will be paid altogether to an account designated by you, from “Yokohama City University”. Please read the separate written information document related to the receipt of Clinical Trial Collaboration Fees.

You may opt out of receiving these payments for whatever reason.

13. Personal Information

In order to check that this study has been conducted properly, study-related personnel may access your medical records that are related to the study (medical charts etc.), even in situations where you prematurely withdraw from this study. Study-related personnel include employees of companies contracted by the Principal Investigator, members of the IRB, officers/employees of the Ministry of Health, Labour and Welfare, as well as individuals contracted by the government to carry out investigations. These individuals are mandated by law to maintain the confidentiality of the contents of records such as medical records that they access, which means that information related to your privacy will not be leaked outside the hospital.

The results of this study may be reported to the company selling the drug that has the

same ingredient as AJG533 (Elobixibat), and may be used as part of the data to be submitted to the government (MHLW). Furthermore, data obtained from this study may be presented in medical journals and conferences. Furthermore, information specific to you in the clinical trial report will use an identifier code that is a combination of numbers and letters, rather than personal information about you such as names and address etc. The list that links your identifier code to your name etc. will be kept and managed in the hospital, in accordance with rules related to the hospital's personal information management. However, information such as the date of birth that is part of the personal information, although mentioned in clinical trial reports for the purposes of checking the clinical trial participation criteria etc., will not be leaked to the outside or be used for purposes other than the study. Sufficient care will be taken to protect your privacy when handling your personal information.

Please understand that, by listening to this explanation and signing this consent form, you will be agreeing to such access to medical records and use of personal information (date of birth, etc.).

14. Your Responsibilities

Please follow the instructions below to ensure your safety, and for us to collect reliable data.

(1) You will be required to fast for 8 hours prior to the blood tests that will be conducted during Week 4, 8, 12, and 16 Visits, as well as at the time of Discontinuation. For this reason, please take the study drugs at the following timing:

• If you will be examined during the morning, please come to the hospital after having taken the study drugs and without eating breakfast.

• If you will be examined during the afternoon, please take the study drugs at the

same timing before breakfast, and come to the hospital without eating lunch. Furthermore, as it is necessary that you fast from 8 hours prior to the blood tests, you may need to come to the hospital without eating breakfast, depending on the timing of your meals.

(2) Please maintain stable diet and normal life (exercise level) during your participation in the study.

(3) If you will seek medical attention at another hospital, please notify the attending doctor that you are participating in a clinical trial. Please also notify the investigator or the Clinical Research Coordinator that you were examined by another doctor. With your permission, we may contact the doctor at the other hospital about your symptoms and medicines taken.

(4) Please comply with all scheduled study visits. (Please notify us in advance if there is a scheduling clash.)

(5) Please discuss with the investigator in advance if you are currently using other medicines (including over-the-counter drugs and health foods), will start using new medicines after beginning study participation, or receive new treatments other than drugs. (Depending on the drug, they may interact with the study drugs and reduce or intensify the effect of the study drugs.)

(6) Please contact the investigator at any time if you notice that your condition is unusual (including bone fractures and accidents).

(7) On study visit dates, please bring your patient diary, remaining study drugs, packets of study drugs and empty study drug sheets with you.

(8) If you are a woman of childbearing potential, please practice contraceptive methods during your participation in the study. Please discuss with your investigator to select a contraceptive method that suits you best. Please notify us immediately if you think that the contraceptive method did not work well, or you think you may have become

pregnant.

(9) Please be sure to notify the investigator if there are any changes to your contact information such as address and telephone number.

15. Disaster Emergency Message Dial during Large-Scale Disasters

We may contact the telephone number that you provide us in advance to check your safety (including family members) in the event of a large-scale disaster. If the communication line stagnates, please cooperate by using the Disaster Emergency Message Dial number “171”.

The Disaster Emergency Message Dial has been established with the intent to be used 30 minutes after the incidence of an earthquake that has a seismic coefficient of 6 or higher, and it is a service that allows you to convey safety from disaster-affected sites using telephones and the Internet.

[How to record on the Disaster Emergency Message Dial “171”]

① Dial “171”, and listen to the guidance

② Dial (1) (Record)

③ Dial your home telephone number (City Code • ○○○○-○○○○).

④ You will be asked to indicate the type of your telephone device (push-button or dial phone).

⑤ Please record your name and contact information according to the guidance provided.

[How to replay a message on Disaster Emergency Message Dial "171"]

① Dial "171", and listen to the guidance

② Dial (2) (Replay)

③ Dial the telephone number of the person you wish to contact (City Code • ○○○○-○○○○).

④ You will be asked to indicate the type of your telephone device (push-button or dial phone).

⑤ The latest message will be played first.

[Broadband message board "web171" for disasters]

① Search Web171 and access the website https://www.web171.jp/

16. Clinical Trial Help Desk

Yokohama City University Hospital

(1) Name of Principal Investigator: Takaomi Kessoku, Assistant Professor

Name of Your Investigator:

Tel.: 045-787-2800

(2) Help Desk: Yokohama City University Hospital

Office of Clinical Trial Management

Tel.: 045-352-7510 (Weekdays from 9:00 to 17:00)

(3) Contact Information at Night-times / Holidays

Tel.: 045-787-2800 (Main Number)

*Please contact the above number at night-times, holidays and at times of emergency, and speak to the on-call doctor of the Department of Gastroenterology.

Attachment 1 **Explanation of Terminology**

No.	Term	Explanation
1)	Liver cirrhosis	A disease in which inflammation occurs in cells of the liver, and repeated repairing of inflammation leads to the liver hardening and losing its function.
2)	Liver cancer	A disease in which liver cells become cancerous.
3)	Chronic constipation	A condition where constipation symptoms such as the reduced frequency of bowel movements and fecal quantity persist for a long period of time.
4)	Hypercholesterolemia	A condition where cholesterol concentration in the blood is high.
5)	Leflunomide	A drug used to treat rheumatoid arthritis.
6)	Active metabolites	New substance(s) generated from the metabolism of another.
7)	Placebo	A mimetic drug that does not contain the active ingredient of the drug, and its appearance is indistinguishable from the active study drug. It is believed that by giving some study participants a placebo, it is possible to more accurately determine how “real” the efficacy and safety of the study drug is.
8)	Liver biopsy	A test that diagnoses a disease by cutting a piece of the liver and examining it.

No.	Term	Explanation
9)	MRI scans	A test that has the patient enter a tube made of a powerful magnet in order to take images of the organs and blood vessels in the body using magnetism.
10)	Liver fat mass	A number that indicates how much fat is on the liver.
11)	Fasting serum LDL-cholesterol	A type of fat found in the blood while fasting.
12)	Dyslipidemia	A condition where levels of lipids such as cholesterol and neutral fats contained in the blood are above certain criteria.
13)	MR elastography	A test where vibration is applied to the liver, and the vibrating liver is pictured by MRI to measure the hardness of the liver.
14)	BMI	This is short for Body Mass Index, which is calculated based on the relationship between body weight and height, and indicates the degree of obesity.
15)	Wilson disease	A disease that is caused by a genetic abnormality, which results in improper excretion of copper, and subsequently its accumulation in the body.
16)	α1-antitrypsin deficiency	A disease where lack of α1-antitrypsin in the body results in various abnormalities in the lungs such as dyspnea and cough.

No.	Term	Explanation
17)	Hemochromatosis	A disease where something causes abnormal increase in iron levels in the body that results in adverse effects on various organs, and destroys cells and tissues to disrupt organ function.
18)	HIV	A virus that infects human immune cells and causes acquired immunodeficiency syndrome (AIDS).
19)	Celiac disease	A disease where an erroneous immune reaction to “Gluten” contained in wheat results in various adverse effects to the small intestines.
20)	Ascites	A disease where more water than usual is retained in the peritoneum that wraps the abdominal organs. Although there are various causes to this, liver cirrhosis and inflammatory diseases make this easier to happen.
21)	Hepatic encephalopathy	Reduced liver function leads to accumulation of unnecessary substances in the blood, and these substances adversely affect the brain to produce symptoms like consciousness disorders.
22)	Esophageal varices	A condition in which the veins on the mucous membrane of the esophagus thickens and appears like a lump. This tends to occur in patients with increased pressure on the “Portal vein” that carries nutrients to the liver, and patients with liver cirrhosis.

No.	Term	Explanation
23)	ALT	An enzyme that is normally present inside the cells. Its level in the blood increases when there is something wrong with the liver.
24)	AST	An enzyme that is normally present inside the cells. Its level in the blood increases when there is something wrong with the liver, heart and muscles.
25)	Abdominal distension	When the stomach feels expanded.
26)	Nausea	A bad feeling that makes you want to vomit.
27)	Bloating	When the stomach swells up due to gas accumulation in the gut.
28)	Dry mouth	Feeling of thirst.
29)	CK	This is short for creatine kinase, which is an enzyme that is found in abundance in the muscles and brain. The level of CK in blood rises when there is inflammation in these tissues.
30)	Epigastric pain	Pain in the pit of the stomach.
31)	Al-P	An enzyme that is normally present inside the cells. Its level in the blood increases when there is something wrong with the liver or bones.

No.	Term	Explanation
32)	LDH	An enzyme that is normally present inside the cells. Its level in the blood increases when there is something wrong with the liver or muscles.
33)	Bilirubin	A pigment released when red blood cells are destroyed. Its level rises in liver diseases.
34)	BUN	A type of waste product of substances used in the body. Its level in the blood and urine rises when there is inflammation in the kidneys, or the kidney functions deteriorate.
35)	Creatinine	A type of waste product of substance used in the body. Its level in the urine rises when kidney functions deteriorate.
36)	Hemoglobin	A protein contained in blood. It has the role of carrying oxygen from the lungs to all over the body.
37)	Hematocrit	This indicates the ratio of volume of red blood cells in the blood. Its level decreases when the patient has anemia.
38)	Rash	When red bumps appear on the skin.
39)	Pruritus	When the skin feels itchy.

No.	Term	Explanation
40)	Gastric ulcer	A condition in which the wall of the stomach is injured. It can cause symptoms like abdominal pain and anemia due to bleeding.
41)	Gingival swelling	The swelling of gums caused by bacterial infection or malnutrition.
42)	Erythema	Red spots forming on the skin.
43)	Oral aphtha	These are generally called stomatitis, and refer to blotches that develop in the mouth.
44)	Serum potassium	A substance called potassium in the blood.
45)	Serum phosphorus	A substance called phosphorus in the blood.
46)	Serum uric acid	A substance called uric acid in the blood.
47)	Vitreous hemorrhage	Bleeding on the inner side of the eye. This may deteriorate the vision.

Attachment 2 **Compensation for Health Injuries**

A Phase II Investigator-led Study to Compare the Efficacy and Safety of Combined Elobixibat and Cholestyramine to that of Placebo, Cholestyramine Monotherapy or Elobixibat Monotherapy for Nonalcoholic Fatty Liver Disease

(Investigator-led Clinical Trial)

Although this clinical trial will be carried out with the utmost care, we have established guidelines and procedures in the event that you suffer health injuries as a result of taking the study drugs or participating in the study.
The purpose of this document is to provide detailed explanations for the contents related to compensation mentioned in the Informed Consent Form. Please keep this document safe along with your copy of the ICF.

If you notice any health injuries such as side effects, please do not hesitate to notify the investigator or a clinical trial collaborator. Treatments and other appropriate measures thought to suit you best will be taken.

1. Compensation for health injuries that occur in this study

(1) Principle of compensation

1) Compensation is to appropriately cover for damages in the event you suffer a health injury, in accordance with the GCP Ordinance (rules stipulated by the government for when clinical trials are conducted), even if the medical institution has no legal liability (when there is no negligence) for the health injury.

2) If you experience a health injury as a result of your participation in this study, you will be compensated based on the guidelines and procedures for compensation.

3) If liability is discovered, you may file a claim for damages. This compensation system does not prevent you from exercising your right to claim damages.

(2) Criteria for compensation

The compensation provided in this study include disability compensation and bereaved family compensation in the event of residual sequelae (Grade 1 to 3) and death, respectively. There will be no payments towards medical expenses and medical allowances in this study.

Health injuries other than sequelae (Grade 1 to 3) and death will be compensated in the form of provision of medical care. As medical expenses incurred at that time will be paid by your health insurance program, you will be asked to pay part of the costs.

(3) Situations that are not compensated for

1) You cannot receive compensation if there is no causal relationship between your health injury and this study.

2) You cannot receive compensation if the hospital, the investigator at this hospital and another third party has legal liability for your health injury, and the responsible party should pay for the damages caused.

3) You cannot receive compensation for health injuries that occurred because of your intentional doing.

(4) Situations when compensation is limited

The compensation payment may be reduced in amount or void altogether if the health injury occurs as a result of your gross negligence (lying or making false reports, failure to follow the instructed dosage and administration, failure to listen to the instructions of the investigator etc.).

2. Compensation procedures

(1) What to do if you experience a health injury

If you experience some form of a health injury as a result of this study, this hospital will provide treatments and take other measures considered most suitable for you.

(2) Request for compensation

Please notify the investigator or the clinical trial collaborator, if you feel you have suffered a health injury, such as side effects etc. The Principal Investigator will determine the causal relationship between the health injury and the study, before explaining to you whether or not you can receive compensation for your health injury.

Please do not hesitate to ask the investigator about any other questions you may have about compensation.

3. Treatment of personal information

Personal information about you collected in the process of providing compensation will be managed and treated appropriately according to the “Personal Information Protection Law”, and will not be used for any purposes other than compensation.

4. Other

Please do not hesitate to contact the investigator, clinical trial collaborator or the Clinical Trial Help Desk listed in the written information document, if you have any questions regarding compensation.

Clinical Trial Protocol No.: YCU-19002
Yokohama City University Hospital Ver1.4
Date prepared: February 20, 2020

Consent Form

Reference No.:（19-466） [Copy for Medical Chart (Physician)]

Herewith I provide my voluntary consent to participate in the clinical trial titled [A Phase II Investigator-led Study to Compare the Efficacy and Safety of Combined Elobixibat and Cholestyramine to that of Placebo, Cholestyramine Monotherapy or Elobixibat Monotherapy for Nonalcoholic Fatty Liver Disease], after being given explanations about this study and having thoroughly understood what was explained to me. I will also receive and store a copy of the written information document and this consent form.

Signature of the Clinical Trial Participant

Date of Consent: __/__/____ Patient Signature：_____

Do you wish to receive burden-relieving payments for study participation? (Yes / No)

Do you consent to providing blood samples for exploratory research? (Yes / No)

Do you consent to providing fecal samples for exploratory research? (Yes / No)

Signature of Physician Handling Informed Consent Process

Date of Explanation: __/__/____ Physician Signature：_____

Signature of Collaborator Providing Supplementary Explanations

Date of Explanation: __/__/____ Collaborator Signature：_____

Date of Issue of Consent Form: __/__/____

Clinical Trial Protocol No.: YCU-19002
Yokohama City University Hospital Ver1.4
Date prepared: February 20, 2020

Consent Form

Reference No.: (19-466) [Copy for Office of Clinical Trial Management]

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Signature of Physician Handling Informed Consent Process

Date of Explanation: __/__/____ Physician Signature：_____

Signature of Collaborator Providing Supplementary Explanations

Date of Explanation: __/__/____ Collaborator Signature：_____

Date of Issue of Consent Form: __/__/____

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Yokohama City University Hospital Ver1.4
Date prepared: February 20, 2020

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Date of Explanation: __/__/____ Collaborator Signature：_____

Date of Issue of Consent Form: __/__/____

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2,20
	2b	All items from the World Health Organization Trial Registration Data Set	2, 20
Protocol version	3	Date and version identifier	2
Funding	4	Sources and types of financial, material, and other support	23
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,23
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	21,23
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	19,20
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9-15
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5, figure1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	18
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	16
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	18
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5-8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	15-17 figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	5, 9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	15
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	15

Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	15
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	15
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	15
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	15-17
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	15-17
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	19, 20
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18-19
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18-19
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18-19
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	19, 20
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	19
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15

Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	19, 20
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	2, 20
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	20
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6, 20
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	15,16,17
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	22,23
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	23
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	20
	31b	Authorship eligibility guidelines and any intended use of professional writers	20
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	23
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	20
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	15-17

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.