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Efficacy and safety of guanabenz acetate treatment for non-alcoholic fatty liver disease: a study protocol for a randomised investigator-initiated phase IIa study

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ABSTRACT

Introduction Non-alcoholic fatty liver disease (NAFLD) is a metabolic syndrome phenotype in the liver and thus obviously associated with metabolic abnormalities, including insulin resistance-related to hyperglycaemic and hyperlipidaemia. The prevalence of NAFLD is increasing worldwide. However, currently, there is no consensus regarding the efficacy and safety of drugs used to treat patients with NAFLD/non-alcoholic steatohepatitis (NASH). Guanabenz acetate, a selective α2-adrenoceptor stimulator used in the treatment of hypertension, binds at a high-affinity constant to a nuclear transcriptional coregulator, helicase with zinc finger 2 (Helz2) and inhibits Helz2-medaited steatosis in the liver; chronic oral administration of guanabenz acetate produces a dose-dependent inhibition of lipid accumulation by inhibiting lipogenesis and activating fatty acid B-oxidation in the liver of obese mice, resulting in improvement of insulin resistance and hyperlipidaemia. Taken all together, guanabenz acetate has a potentially effective in improving the development of NAFLD/NASH and metabolic abnormalities. In this randomised, open label, parallel-group, phase IIa study, we made attempts to conduct a proof-of-concept assessment by evaluating the efficacy and safety of guanabenz acetate treatment in patients with NAFLD/NASH.

Methods and analysis A total of 28 adult patients with NAFLD or NASH and hypertension complications meeting the inclusion/exclusion criteria will be enrolled. Patients will be randomised to receive either 4 or 8 mg guanabenz acetate (n=14 per group). Blood tests and MRI will be performed 16 weeks after commencement of treatment. The primary endpoint will be the percentage reduction in hepatic fat content (%) measured using MRI-proton density fat fraction from baseline by at least 3.46% at week 16 after treatment initiation.

Ethics and dissemination Ethics approval was obtained from the Ethics Committee of Yokohama City University Hospital before participant enrolment (YCU021001). 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. Participants wishing to know the results of this study will be contacted directly on data publication.

Trial registration number This trial is registered with ClinicalTrials.gov (number: NCT05084404).

Protocol version V.1.1, 19 August 2021.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study is the first randomised, open-label, phase IIa trial to determine the efficacy and safety of guanabenz acetate treatment for non-alcoholic fatty liver disease.
⇒ This study will use non-invasive technology (MRI-based proton density fat fraction) to assess fatty liver content (%) as the primary endpoint for guanabenz acetate treatment of fatty liver diseases.
⇒ Secondary endpoints will include lipid classes, disease susceptibility genes, various fibrosis markers, endocrinology and inflammation.
⇒ The limitations of the study include single-centre trial, small sample size, open-label, relatively short-treatment period and no liver biopsy.

INTRODUCTION

Non-alcoholic 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 metabolic syndrome and is often associated with obesity, diabetes mellitus, dyslipidaemia, hypertension and other disorders. The prevalence of NAFLD is increasing worldwide; in Japan, it increased from 12.9% in 1994 to ~34.7% in 2000.[1] NAFLD is classified as non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH), which includes inflammation and a progressive disease associated with liver

cancer or cirrhosis, affecting 10%–20% of patients.[2] As a treatment method, diet and exercise therapy with low-calorie diet are effective, and it has been reported that weight loss improves liver function and liver histology.[3] In cases of concomitant hypertension, treatment with angiotensin II receptor blockers reportedly reduces inflammation and fibrosis in liver tissue.[4] However, there is no consensus on the efficacy of any drugs for NAFLD/NASH, and none are covered by insurance—globally, including in Japan.

Guanabenz acetate has a selective α2-adrenergic receptor-stimulating effect. It is used safely as a therapeutic agent for essential hypertension because it acts on the central nervous system to reduce efferent sympathetic nerve activity and lowers blood pressure by blocking nerve transmission at sympathetic nerve endings.[5] In recent years, separate from its afore-mentioned effects on the nervous system, guanabenz acetate has also been described to bind at a high-affinity constant to helicase with zinc finger 2 (Helz2; also known as peroxisome proliferator-activated receptor-gamma (PPARγ) DNA-binding domain interacting protein 1), which is a transcriptional coregulator that regulates gene expression at the promoter level of the target gene, in conjunction with certain nuclear transcription factors. Helz2 functions as a metabolic sensor and is thought to act as a coactivator by binding to the DNA-binding domain of the PPARγ nuclear transcription factor, which acts as a master regulator of metabolic regulation. Increased expression of Helz2 obviously produces fatty liver and insulin resistance in obese mice. Interestingly, the significant increase in hepatic Helz2 gene expression has been observed in obese patients with fatty liver as compared with those without fatty liver. Binding of guanabenz acetate to Helz2 at a high-affinity constant causes inhibition of Helz2 activity in the liver. In a mouse model of human obesity, chronic oral administration of guanabenz acetate causes a dose-dependent inhibition of hepatic lipids accumulation by inhibiting expression of Scd1, a limited enzyme, to reduce lipogenesis and activating expression of Cpt1a, a gate keeper enzyme, to enhance mitochondrial fatty acid B-oxidation in the liver, resulting in reducing insulin resistance, hyperglycaemic, fatty liver cell count and blood lipid (low-density lipoprotein–cholesterol concentration.[6] Eventually, administration of guanabenz acetate leads to stimulation of energy expenditure to significant attenuation of obesity, but hyperphagia remains unaffected. Therefore, administration of guanabenz acetate has a potential ability in improving development of fatty liver diseases (NAFLD and NASH) associated with insulin resistance,

Therefore, in our study, we aim to investigate the efficacy and safety of guanabenz acetate treatment in patients with NAFLD/NASH.

METHODS AND ANALYSIS

Trial design

The Standard Protocol Items for Randomized Trials statement and its checklist were followed to prepare the study protocol. This trial was designed as a single-centre, randomised, open-label, parallel-group, investigator-initiated study to investigate the efficacy and safety of either 4 or 8 mg guanabenz acetate tablets. The actual study period is from 29 October 2021 to 30 June 2023. The study protocol and informed consent form are shown in online supplemental documents 1 and 2. All treatments will be administered orally two times daily for 16 weeks to patients with NAFLD. The experimental groups will be as follows: the 4 mg group (4 mg guanabenz acetate) and the 8 mg group (8 mg guanabenz acetate) (figure 1). This clinical is a clinical phase IIa study aimed at confirming the proof-of-concept (POC) of guanabenz acetate therapy. We plan to examine the targets and stage in the next phase. MRI will be performed at baseline and 16 weeks after intervention, and the data will be evaluated by a blinded independent liver specialist (KI). The study plan involves recruiting 28 adult patients with NAFLD/NASH from the Yokohama City University Hospital cohort.

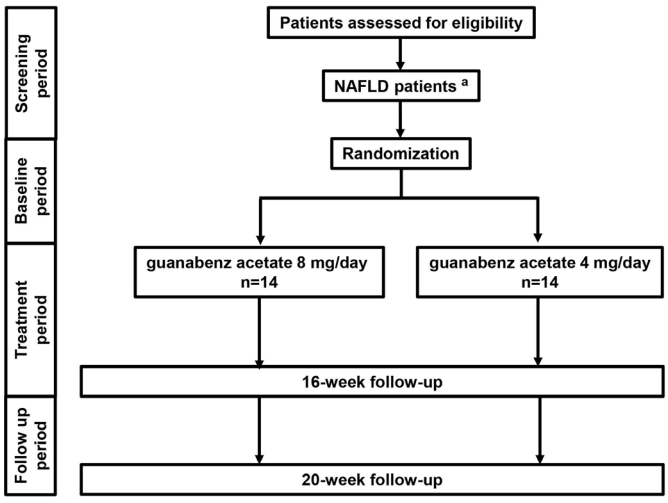

Figure 1 Study design. [a]N=28 enrolled. NAFLD, non-alcoholic fatty liver disease.

Study endpoints and rationale

The primary endpoint will be the percentage of patients in whom the fatty liver content (%) measured using MRI-proton density fat fraction (PDFF) at 16 weeks decreases by 3.46% or more from baseline among patients receiving either 4 or 8 mg guanabenz acetate therapy (table 1). To evaluate hepatic fattening in NAFLD, using MRI-PDFF evaluation is considered appropriate for the pathological condition.

Recent reports have indicated that MRI-PDFF is superior to controlled attenuation parameter in the diagnostic assessment of liver fattening.[7] Liver biopsy is commonly used to assess hepatic fat mass. However, it is unsuitable for monitoring owing to its invasiveness. In contrast, image evaluation using MRI-PDFF has been indicated as stable and highly reproducible. In addition, because the judgement of fat deposition depends on the collection

Table 1 Study endpoints

Primary endpoint	Secondary endpoints	
Efficacy endpoint	Efficacy endpoint	Safety endpoint
Percentage of patients with ≥3.46% decrease in liver fat content measured using MRI-PDFF at 16 weeks from baseline	Amount and rate of change at 16 weeks from baseline in the following parameters: 1. Percentage of patients where the liver fat content (%) measured using MRI-PDFF at 16 weeks decreases by ≥3.46% from baseline for either the 4 or 8 mg group 2. Liver fat content measured using MRI-PDFF 3. ALT, AST and γ-GTP 4. Weight 5. Blood lipids (chylomicron cholesterol, chylomicron triglyceride, lipoprotein–cholesterol, LDL triglyceride, VLDL cholesterol, VLDL triglyceride, free cholesterol, apoprotein A1, apoprotein B, adipsin, free fatty acid) 6. HOMA-IR 7. Liver hardness (MRE) 8. Fibrosis markers (ELF Score, FIB-4)	Occurrence rate of adverse events
	Search for new markers related to liver disease and metabolic syndrome	

All objectives will be compared between 4 and 8 mg guanabana acetate therapies.
ALT, alanine transaminase; AST, aspartate transaminase; ELF, enhanced liver fibrosis; FIB-4, fibrosis-4; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; MRE, magnetic resonance elastography; MRI-PDFF, MRI-based proton density fat fraction; VLDL, very-low-density lipoprotein; γ-GTP, γ-glutamyl transpeptidase.

site of the liver biopsy, MRI-PDFF, which enables easy quantitative evaluation, is considered appropriate.[8–11]

The 24-week MOZART (Magnetic Resonance Imaging and Elastography in Ezetimibe Versus Placebo for the Assessment of Response to Treatment in NASH) trial explored the correlation between histological changes by liver biopsy and hepatic fat content using MRI-PDFF to examine the effect of ezetimibe on NASH. The trial reported an estimated cut-off point of −3.46% for changes in liver fat content from baseline that optimally distinguished between histological responders and non-responders.[12] Patients with ≤3.46% change in hepatic fat content as measured using MRI-PDFF were 4.3 times more likely to be true histological responders than false positives.

The purpose of this clinical trial is to confirm POC. Once POC is established, we plan to confirm the dose setting and treatment period in the next phase and verify efficacy by evaluation, including liver biopsy (confirmation of fibrosis).

Our secondary endpoint will determine the amount and rate of change from baseline (table 1) to 16 weeks. Other variables to be monitored include adverse events (AEs), standard laboratory analysis results, physical examination results, vital signs and compliance rate. Physical assessment will be performed and evaluated at Yokohama City University using standard procedures.

Rationale for treatment dose, mode and duration

To analyse the efficacy of guanabenz acetate, approved doses of 4 and 8 mg for essential hypertension will be used. The doses were set according to clinical data showing that a starting dose of 8 mg ensures sufficient safety. Therefore, the interview form for the medicinal product also shows that for the phase II pilot study, the antihypertensive effect of guanabenz acetate is modest and without significant difference in the incidence of side effects between the starting doses of 4 and 8 mg. After confirming the POC in this study, we plan to investigate the appropriate dose of the drug in a phase IIb study.

Globally, short-term clinical trials for NAFLD (eg, NCT02913105: safety, tolerability, pharmacokinetics and efficacy of LMB763 in patients with NASH; NCT02927314: a study of the efficacy and safety of CF102 in the treatment of NAFLD) have a minimum dosing period of 12 weeks. No significant difference was found in PDFF in a 12-week study, with PDFF as the primary endpoint.[12] However, in this study, the rate of change in hepatic fat mass using image evaluation based on MRI-PDFF will be the primary endpoint. Therefore, it is considered appropriate that an administration period of 16 weeks be used to confirm the efficacy of this drug.

Drug supply

This clinical trial will be open, and the patient registration centre, doctors and patients will be informed of the results of the allocation. The tablets (2 mg) of guanabenz acetate to be used are manufactured and supplied by Toyo Pharmaceutical Kasei (Tokyo and Osaka). These drugs are prescribed by the physician and provided by the patient registration centre (personally dispensed by the pharmacy manager).

Sample size estimation

As described in the setting basis of 'study endpoints and rationale,' the odds of the patients whose liver fat content decreases by less than 3.46% being true histological responders are ~4.3 times greater than that for them being false positives. For this cut-off value, the true positive probability is 0.59, and the false positive probability is 0.25, and the following relationship (Equation 1) holds. Therefore, when the probability that there exists a true

histological responder is 0, then there is a 0.25 probability that the change in liver fat content from baseline is ≤–3.46%.

$$\theta = (a - b) \times q + b = (0.59 - 0.25) \times q + 0.25 \quad (1)$$

θ: Probability of the amount of change in liver fat content from baseline ≤–3.46%.

q: Probability of existence of a true histological responder.

a: True positive probability.

b: False positive probability.

This trial is designed to explore the minimal potential for efficacy in planning a placebo-controlled study in the next phase. That the probability of existence of a true histological responder is greater than 0 can be examined using non-invasive means by showing a>0.25 proportion of patients with a change in liver fat content from baseline ≤–3.46%. Therefore, assuming that a binomial distribution is followed for the event in which the change in liver fat content from baseline ≤–3.46%, we decided to set a sample size that can reject the null hypothesis H_0:θ≤0.25 using a one-sided test at the 5% level. As a concrete alternative hypothesis for setting the power, we assume H_0:θ=θ_1=0.5, which corresponds to the existence probability of 0.75 for a true histological responder. The sample size required to obtain 80% power is 28 cases.

In this study, the main concern is the total number of active drug administration cases. Only half the required sample size will be assigned to each dose group (4 and 8 mg).

Eligibility

The physicians will enter consenting 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 their informed consent. This is because (1) the legal 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 AEs. If no eligibility issues are identified, the investigator or subinvestigator and investigative staff will enter the necessary information into the electronic data capture (EDC) system for enrolment. Enrolment will then be completed by assigning the patient an enrolment number.

Randomisation and masking

The principal investigator or subinvestigator will obtain written consent from the candidate patient. Thereafter, the principal investigator or subinvestigator will enter the patients in the screening list, assign a patient identification code to each patient who has provided their consent and confirm their eligibility according to the selection and exclusion criteria. In the absence of any eligibility concern, the principal investigator or subinvestigator and clinical trial collaborators will enter the necessary information into the EDC system, register at the day to prescribe and issue a case registration number. Registration will be completed using this number. At the time of enrolment, patients will be assigned to one of the two groups (4 or 8 mg/day guanabenz acetate) at a ratio of 1:1 using the allocation table prepared using the substitution block method. Given the thorough screening criteria, no adjustment factors should be required. The applicable allocation number will be issued via the EDC system. The treatment assignments are not fully masked to the patient and physician.

Keycode break

Not applicable.

Harm and AE monitoring

AEs are any unwanted or unintended side effects, including abnormal laboratory test values or abnormal vital signs, symptoms or illness that occur during the trial. The causal relationship with the investigational drug does not matter. The principal investigator or subinvestigator will assess the severity of the AEs. Any AE that meets any of the following criteria will be considered a serious AEs (SAE): death, life-threat, hospitalisation requirement or prolonged hospitalisation for treatment, disability, disability threat, other serious conditions, congenital disease or anomaly in offspring. If an SAE occurs, the principal investigator or subinvestigator will appropriately treat the SAE and immediately report the details to the hospital director as well as the study drug supplier.

Study procedures

The principal investigator or subinvestigator will conduct observations, inspections and surveys according to the descriptions provided in table 3. If a blood sampling test is to be performed at the time of visit, the patient should fast for 8 hours before blood collection. Blood/stool sampling will be collected and stored for exploratory analysis of genes (single nucleotide polymorphisms; patatin-like phospholipase domain containing 3; transmembrane 6 superfamily member 2), fibrosis and inflammation on obtaining additional consent. The principal investigator or subinvestigator or clinical trial collaborators will provide prescription guidelines to the patients when the drug of investigation is delivered. If a dosing time is accidentally missed, any remaining medicine and an empty sheet of paper would need to be brought on the next visit. The principal investigator or subinvestigator or clinical trial collaborator must recover any unconsumed drugs from the patient. Furthermore, if a dose is missed, patients could take it at least 6 hours before the next dose. The drug should be returned to the investigational drug administrator. If the unconsumed drug cannot be recovered, the reason should be provided in the medical record.

Concomitant treatment

The following drugs will be prohibited from concomitant use from the time of consent acquisition to the treatment period: ursodeoxycholic acid, guanabenz analogues

Table 2 Patient inclusion and exclusion criteria

	Inclusion criteria	Exclusion criteria
1	Patients fully informed about the study and provided written consent	Pregnant, lactating, potentially pregnant women or patients who do not agree to contraception during the trial period
2	Patients ≥20 years of age ≤ 75 years of age at the time of providing consent	Patients who have taken guanabenz acetate within 16 weeks prior to screening or who have participated in other clinical studies (observational studies are excluded)
3	Patients diagnosed with essential hypertension and whose systolic blood pressure at the time of screening is ≥130 mm Hg and/or diastolic blood pressure≥85 mm Hg (according to the diagnostic criteria for metabolic syndrome)	Patients with the following laboratory test values: 1. ALT>430 IU/L (males) or >240 IU/L (female); or AST>300 IU/L (males and females) 2. PT-INR≥1.5 (excluding anticoagulant therapy) 3. Total bilirubin value>2.0 mg/dL (excluding definitive diagnosis of Gilbert syndrome) 4. Platelet count<8.0×10^4/μL 5. eGFR<45 (calculated by body surface area correction: standardised eGFR)
4	Patients diagnosed with NAFLD/NASH who meet criteria (1) or (2): 1. Patients diagnosed with NAFLD who meet the three criteria: 1. Diagnostic imaging or histological evidence of fatty liver 2. Alcohol intake<30 g/day for men and <20 g/day for women for ≥12 consecutive weeks 1 year before screening 3. Absence of other factors that cause fattening or chronic liver disease 2. Patients with a *definitive diagnosis of NASH by biopsy within 32 weeks before screening	Patients with a history of acute or chronic liver disease other than NAFLD/NASH and complications such as: 1. Hepatitis B (defined as HBsAg positive at the time of screening) or hepatitis C (defined as HCV antibody positive at the time of screening). However, anti-HCV antibody-positive patients who are judged negative for HCV-RNA can be registered if they can be confirmed to be negative for at least 1 year before screening 2. Patients with autoimmune hepatitis 3. Patients with primary biliary cholangitis, primary sclerosing cholangitis, Wilson's disease, α1-antitrypsin deficiency, haemochromatosis or iron overload, drug-induced or alcoholic liver disease, or a history of known biliary atresia 4. Patients wit5. Patients with the following laboratory test values
5	Patients with MRI-PDFF liver fat mass≥8% at screening	Patients with allergies to guanabenz acetate
6	Patients with MRE value≤3.6 kPa at screening	Patients with liver failure or cirrhosis
7	Patients with a BMI≥25 kg/m^2 at the time of screening	Patients with a history of HIV infection
8	Patients receiving diet or exercise therapy 12 weeks before screening, with no improvement	Patients with findings of portal hypertension (complications: ascites, hepatic encephalopathy, varicose veins, splenomegaly)
9	Patients willing to maintain stable diet and physical activity during the clinical trial	Patients with a history of NAFLD-related drugs (amiodarone, methotrexate, systemic glucocorticoids, tetracycline, tamoxifen, higher doses of oestrogen, anabolic steroids or valproic acid used for hormone replacement) or other hepatotoxins for at least 4 weeks prior to screening
10		1. Patients who have used the following drugs: 1. Insulin, GLP-1 receptor agonists, SGLT2 inhibitors or thiazolidine 12 weeks before screening 2. Ursodeoxycholic acid or vitamin E 12 weeks before screening 3. Dyslipidaemia or antihypertensive drugs whose doses were changed 12 weeks before screening 4. Oral diabetes treatment drug (DPP-4 inhibitor, SU preparation, α-glucosidase inhibitor, metformin) whose doses were changed 12 weeks before screening 5. Drugs known to have a significant effect on body weight (including over-the-counter drugs for weight loss) 12 weeks before screening 6. Central nervous system depressants (barbital, sodium thiopental, morphine hydrochloride hydrate, brotizolam, diazepam, etc)
11		Patients with 10% wt change 24 weeks before screening
12		Patients scheduled to undergo surgery after obesity surgery (such as gastroplasty and Roux-en-Y gastric bypass surgery) or during the trial period
13		Patients with a history of type 1 diabetes
14		Patients with HbA1c>9.5% at screening or with uncontrolled type 2 diabetes

Continued

Table 2 Continued

Inclusion criteria	Exclusion criteria
15	Patients with hyperthyroidism or hypothyroidism or screening results showing thyroid dysfunction. However, for hypothyroidism, registration is possible if thyroid replacement therapy is received 12 weeks before screening, and the test values are stable
16	Patients with a history of NYHA class III or IV heart failure due to factors other than hypertension
17	Patients with history of myocardial infarction, unstable angina, percutaneous coronary intervention, coronary artery bypass grafting, stroke or major surgery 24 weeks before screening
18	Patients with a history of substance abuse
19	Patients with malignant tumours. However, patients who have undergone radical surgery, have completed chemotherapy/radiation therapy and are undergoing hormone therapy can be registered
20	Patients with known intolerance to MRI or patients contraindicated for MRI examination
21	Other patients who the principal investigator or subinvestigator deems inappropriate for being enrolled in this clinical trial

*The definitive diagnostic criteria for NASH are defined as a fibrosis stage in liver biopsy in the evaluation using the 'NASH CRN criteria' by an F1–F3 pathologist and an NAS≥4 points (each item has one or more points): (1) Fattening (0–3 points), (2) Balloon-like swelling (0–2 points), (3) Inflammation in the lobules (0–3 points).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRN, clinical research network; DPP-4, dipeptidyl peptidase 4; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide-1; HbA1c, haemoglobin A1c; HBsAg, hepatitis B surface; HCV, hepatitis C virus; HIV, human immunodeficiency virus; MRE, magnetic resonance elastography; MRI, magnetic resonance imaging; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; NYHA, New York Heart Association; PDFF, proton density fat fraction; PT-INR, prothrombin time-international normalised ratio; RNA, ribonucleic acid; SGLT2, sodium-glucose cotransporter 2; SU, sulfonylurea.

(clonidine, methyldopa), thiazolidine, glucagon-like peptide-1 receptor agonist, sodium-glucose cotransporter 2 inhibitor, insulin, central nervous system depressants (barbital, sodium thiopental, morphine hydrochloride hydrate, brotizolam and diazepam), vitamin E, NAFLD-related drugs (amiodarone, methotrexate, systemic glucocorticoids, tetracycline, tamoxifen, higher doses of oestrogen, anabolic steroids or valproic acid than used for hormone replacement) or other hepatotoxins, and drugs that significantly affect body weight (including over-the-counter weight loss drugs).

When the following drugs and therapies are used in combination during the treatment period, prescribed conditions must be adhered to. The following drugs may be used concomitantly only if the dose is kept constant from 12 weeks before screening and the drug is continuously used. The dose also may not change until the end of the investment drug administration. These drugs include antihypertensive drugs, drugs to treat dyslipidaemia and drugs to treat diabetes (DPP-4 inhibitor, SU preparation, α-glucosidase inhibitor, metformin). If hypertension symptoms worsen in patients using antihypertensive drugs, only calcium antagonists may be additionally administered.

Criteria and procedure for withdrawal from the study

The principal investigator or subinvestigator should terminate the participation of a patient enrolled in a clinical trial if any of the following applies: (1) withdrawal from the clinical trial is requested by the patient; (2) it is found after registration that the patient does not meet the inclusion criteria or conforms to one or more exclusion criteria; (3) drugs or therapies whose concomitant use is prohibited are being administered; (4) it is difficult to continue the clinical trial owing to the occurrence of AEs or for other reasons; and (5) continuation of the clinical trial is judged to be inappropriate by the principal investigator or others.

Efficacy evaluation

The primary efficacy endpoint will be the percentage of patients in whom the liver fat content (%) measured using MRI-PDFF at 16 weeks decreases by 3.46% or more from baseline (%). The secondary endpoints are presented in table 1. MRI-PDFF/magnetic resonance elastography (MRE) will be performed by an independent liver specialist blinded to the treatment.

Safety assessments

The occurrence rate of AEs will be monitored during each patient visit from the time of treatment initiation until the 4-week follow-up period.

Population analysis

The set of patients to be analysed will be determined before logging the data of each patient defined as follows: The modified intention-to-treat, which is the full analysis set (FAS) and per-protocol set (PPS), will be used to assess primary efficacy. The FAS will include all patients who were randomised, except those who met any of the following

Table 3 Schedule for observations, tests and assessments

	Consent acquisition	Screening	Treatment period					Follow-up
		V1	V2/randomisation	V3	V4	V5	V6	V7/EOT	V8
Study week		week −8 to day −1	day −1	week 2	week 4	week 8	week 12	week 16	4 weeks post administration
Visit window			–	±3 day	±7 day	±7 day	±7 day	±7 day	±7 day
Consent acquisition	○								
Selection criteria		○	○						
Patient background		○							
Serological test*		○							
X-ray of the chest		○							
Electrocardiogram		○							
Physical examination†		○	○					○	
Vital signs‡		○	○	○	○	○	○	○	○
Subjective/objective symptoms			○	○	○	○	○	○	○
Pregnancy test§			○					○	
MRI¶		○						○	
Liver biopsy		Δ							
Randomisation			○						
Haematology/urine test§§		○	○**					○	○
Endocrinological examination		○							
Biochemical test 1			○**		○	○	○	○	○
Biochemical test 2††			○**					○	
Other‡‡			○					○	
Somatic cell genetic test			•						
Providing drugs			○		○	○	○		
Checking the medication status				○	○	○	○	○	
Survey of combination drugs		○	○	○	○	○	○	○	
Investigation of adverse events				○	○	○	○	○	○

○Indicates implemented.
ΔIndicates information is collected for cases with liver biopsy results (within 32 weeks prior to screening).
•Indicates genetic testing is essential.
*Contains hepatitis B antigen, hepatitis C virus (HCV) antibody and HCV-RNA.
†Includes height (V1 only) and weight. BMI (V1) calculated based on height and weight.
‡Vital signs include blood pressure, pulse rate and axillary body temperature.
§For women of childbearing potential, a urine pregnancy test will be performed on V2 and V7.
¶MRI will be used to measure magnetic resonance elastography and liver fat (proton density fat fraction). Patients terminating before V7 (week 16) should undergo MRI at the end of treatment after completing at least 4 weeks of treatment.
**If there are data within 4 weeks, it can be substituted.
††Refer to table 4, Clinical laboratory items.
‡‡Refer to table 4, Clinical laboratory items.
§§Refer to table 4, Clinical laboratory items.
MRI, magnetic resonance imaging.

criteria: (1) cases of serious clinical trial protocol violations (violations of consent acquisition, serious violations of clinical trial procedures, etc); (2) cases in which the investigational drug has never been administered; and (3) cases in which no endpoints related to efficacy were measured. The PPS will be a subpopulation of the FAS excluding cases with clinical trial protocol violations, such as ex post facto findings of inclusion criteria violations or the use of drugs or treatments whose concomitant use is prohibited. The safety analysis set will be used for safety

Table 4 Clinical laboratory items

Biochemical test 1 (on an empty stomach) (Screening, every visit, follow-up, termination)	Biochemical test 2 (on an empty stomach) (at the time of screening, V2, V7, termination)	Others (V2, V7/at termination)
Albumin ALT Alkaline phosphatase Amylase AST Blood urea nitrogen Chlorine Creatinine Estimated glomerular filtration rate* (during screening) γ-GTP Lactate dehydrogenase Potassium Sodium Calcium Total bilirubin Total protein Uric acid	HDL-C LDL-C Non-HDL-C* TC TG Glucose HbA1c Insulin HOMA-IR*	(Inflammation) High-sensitivity C reactive protein Ferritin TNF-a Interleukin 6 CK18/M30 Endotoxin LBP Endotoxin activity (Endocrine) C-peptide Total GLP-1/active GLP-1 Leptin Adiponectin (Fibrosis) hyaluronic acid PIIIP TIMP-1 M2BPGi Type 4 collagen 7 s ELF Score 3 Fibrosis-4* (Fat) Chylomicron cholesterol Chylomicron triglyceride Lipoprotein cholesterol LDL triglyceride VLDL cholesterol VLDL triglyceride Free cholesterol Apolipoprotein A1 Apolipoprotein B Adipsin Free fatty acid (Others) TMAO
Haematological examination/coagulation (During screening, V2, V7, follow-up, termination)	Urinalysis (Screening, V2, V7, termination, follow-up)	
Haematocrit Haemoglobin Platelet count Number of red blood cells White blood cell count and white blood cell fraction (neutrophils, eosinophils, basophils, lymphocytes, monocytes) International normalised ratio	Latent blood Urine sugar pH Urine protein Specific gravity Urobilinogen Pregnancy test† (At V2, V7, at termination)	
Somatic cell genetic test (V2)	Endocrinological examination (at the time of screening)	Serological test (at the time of screening)
PNPLA3 TM6SF2	Free thyroxine (FT4) Free triiodothyronine (FT3) Thyroid stimulating hormone	HBs antigen HCV antibody‡

*According to the calculation formula.
†Postmenopausal is defined as a condition without medical causes and no menstruation for more than 12 months.
‡Perform HCV-RNA test if HCV antibody is positive or if hepatitis C is present in the past.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; ELF, enhanced liver fibrosis; GLP-1, glucagon-like peptide-1; HbA1c, haemoglobin A1c; HBs, hepatitis B; HCV, hepatitis C virus; HDL-C, high-density lipoprotein–cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LBP, lipopolysaccharide-binding protein; LDL-C, low-density lipoprotein–cholesterol; M2BPGi, mac2 binding protein glucosylation isomer; PNPLA3, Patatin-like phospholipase domain containing 3; PIIIP, procollagen III peptide; TC, total cholesterol; TG, triglyceride; TIMP-1, tissue inhibitor of metalloproteinases-1; TMAO, trimethylamine N-oxide; TM6SF2, transmembrane protein 6 superfamily member 2; TNF-α, tumor necrosis factor-α; VLDL, very-low-density lipoprotein; γ-GTP, γ-glutamyl transpeptidase.

assessment and will include all cases in which the investigational drug was administered at least once.

Statistical analysis

The main analysis will be conducted on the FAS. A point estimate will be used to calculate the proportion of patients whose 'liver fat content (%) measured using MRI-PDFF at 16 weeks decreased by 3.46% or more from baseline' with a 90% Clopper-Pearson CI. The following hypothesis test will then be performed: If the lower limit of the 90% Clopper-Pearson CI is >0.25, we reject the null hypothesis H0: $\theta \geq 0.25$ at the 5% level and conclude that $\theta < 0.25$ (binomial test). Here, θ is the probability that the amount of change in liver fat content from baseline $\leq -3.46\%$. For each secondary endpoint, each group will be summarised using descriptive statistics and parallel-group comparison using t-tests. In each group, the number and proportion of AEs will be calculated according to the event and severity. When performing a test or interval

estimation, the significance level is 5% (two sided) and the confidence coefficient is 95% unless otherwise specified. Multiplicity is not considered for the test and the interpretation of confidence intervals.

Amendment of the clinical trial protocol

Those who conduct their own clinical trials amend clinical trial protocol and case report form samples as necessary, when non-administrative matters of the clinical trial apply. Institutional review boards may approve the amended form samples given the following reasons:

1. When important information on matters such as those related to the quality, efficacy and safety of investigational drugs must be updated for the proper conduct of clinical trials.
2. When medically unavoidable circumstances warrant a change in the clinical trial protocols.
3. When the head of the implementing medical institution gives instructions for correction based on the opinion of the institutional review board.

Conclusion, termination or suspension of the clinical trial

After the clinical trial, the principal investigator will inform the head of the implementing medical institution that the clinical trial has ended; the head will also be provided a written summary of the clinical trial results. Subsequently, the institutional review board will be promptly notified in writing that the head of the implementing institution has received the report; the board will also be provided the clinical trial results outlined from the report submitted by the principal investigator.

In case of clinical trial termination or suspension, the clinical trial conductor will promptly send a written report detailing the termination or suspension and the reason to the director of the implementing medical institution and the regulatory agency. The clinical trial conductor may terminate or suspend the clinical trial under the following circumstances:

1. Ethically or medically unavoidable circumstances occur, such as ensuring the safety of the participants.
2. The clinical trial is deemed insignificant.
3. The principal investigator or implementing medical institution has hindered the proper clinical trial by violating the Good Clinical Practice (GCP) Ministerial Ordinance, clinical trial protocol or various procedure manuals (except for other medically unavoidable cases, to avoid urgent danger).

Interim analysis

Not applicable.

Data management, central monitoring and audit

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

Study flow and schedule of enrolment, interventions and assessments

A flowchart of the study is shown in figure 1. The study schedule is listed in table 3.

Clinical trial quality control and assurance

Those who conduct clinical trials by themselves shall establish an audit department independent of the clinical trial department and conduct audits at an appropriate time to guarantee the quality of the clinical trial. On request from the auditor, the principal investigator and the head of the implementing medical institution will provide the necessary information to the institutional review board, including all clinical trial-related records, such as source documents. The auditor will confirm whether the quality control of the data has been performed according to the GCP, standard work procedure manual, clinical trial protocol and other predetermined plans. The person in charge of the audit confirms and approves the report from the auditor.

Patient and public involvement

In this randomised controlled trial, patients will be involved in the recruitment and conduct of the study. In particular, the development of the research question and outcome measures will be based on the priorities, experiences and preferences of patients. The results of this study will be disseminated by email to participants who indicate interest in the results. The burden of intervention will be assessed by patients before the commencement of the trial; patient satisfaction with the treatment will be assessed as a part of the postintervention 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 GCP standards. The study protocol and relevant supporting data were approved on 19 August 2021 by the Institutional Ethics Committee before participant enrolment (YCU021001). The trial results will be reported in accordance with the Consolidated Standards of Reporting Trials 2010 guidelines. This trial has been registered with the ClinicalTrials.gov registry and will be overseen by an external monitor and clinical research organisation. Written informed consent (see online supplemental document 1) for study participation will be obtained from all enrolled participants. 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 trial results of the investigators. Authorship will be ascribed in accordance with the guidelines of the International Committee of Medical Journal Editors.

Health damage compensation and insurance

If any health hazard occurs to a participant as a result of this clinical trial, the principal investigator, among others, will provide treatment and take other necessary measures for the participant. Those who conduct clinical trials by themselves shall establish a procedure manual, take measures such as taking out insurance to compensate for the health damage sustained by the participant in relation to the clinical trial, respond to the health hazards of the participants in accordance to the procedure manual and take out the insurance necessary to prepare for health damage compensation. If the health damage is caused by medical malpractice, the implementing medical institution will take out the insurance and observe the other necessary measures.

DISCUSSION

This POC study is proposed to evaluate the efficacy of guanabenz acetate in patients with NAFLD/NASH. Since the investigational drug being evaluated is a therapeutic drug for hypertension and the frequency of NAFLD complications in hypertensive patients is high, NAFLD patients with NASH and hypertension should serve as an appropriate target group to test the medical efficacy of the treatment.

Guanabenz acetate binds to Helz2, which acts on the liver, and is expected to suppress liver fat accumulation, producing an antiobesity effect. Simple steatosis in the liver increases the risk of mortality.[13] Therefore, fatty liver content (%) is appropriate as the primary endpoint of the phase IIa study. Based on the results of this study, liver fibrosis may also be considered as a primary endpoint of the phase IIb study.

In some well-known trials, the primary endpoints included liver histology, which was evaluated using liver biopsy specimens.[14 15] Liver histology endpoints, such as the complete resolution of NASH, are considered surrogates for preventing cirrhosis in that they potentially predict clinical benefit. However, liver biopsy can pose limitations in terms of costs, possible risks, interobserver and intraobserver bias, sampling errors and healthcare resource utilisation.[16 17]

In recent years, MRI techniques have advanced significantly, and MRE can be used to diagnose hepatic adiposity and hepatic fibrosis with very high sensitivity and specificity.[18 19] It is also possible to quantify adiposity using MRI by measuring PDFF using the iterative decomposition of water and fat based on echo asymmetry and least squares estimation sequencing (IDEAL IQ).[7 20] MRE and MRI-PDFF can be performed simultaneously in a single imaging session, and the results can be combined to assess hepatic adiposity and fibrosis. Being non-invasive, assessment of hepatic fibrosis and hepatic adiposity using MRI has the potential to replace liver biopsy in clinical practice. Furthermore, MRI-PDFF quantification of hepatic fat mass is sufficiently sensitive to be used for quantitative fat assessment in clinical trials of NASH.[7 18] Patel *et al* used paired data from MRI-PDFF and liver histology to show that absolute changes in hepatic fat mass of –4.1% and a relative change of –29.3% were associated with histological improvement, that is, a decrease of at least two points in NAFLD activity score and a decrease of one point each in fat deposition and ballooning.[12] We chose MRI-PDFF as an alternative to liver histology to assess the amount of fat in the liver.

This study has several strengths. First, this is the first clinical trial to focus on the efficacy of guanabenz acetate treatment in patients with NAFLD. Second, MRI will be taken according to a standardised protocol and processed under the supervision of a liver radiologist blinded to the study. Finally, exploratory endpoints such as lipid classes, disease susceptibility genes, various fibrosis markers, endocrinology and inflammation will be measured. However, this study also has the following limitations. First, it will be carried out in a single centre and open label. Second, the sample size is relatively small. Third, the duration of treatment will be relatively short. Finally, no liver biopsy will be performed. For the primary endpoint, instead of histological assessment, a cut-off value for liver fat content using MRI-PDFF was established with reference to the secondary analysis of MOZART placebo-controlled randomised study.[12] It is possible that the change in steatosis is small to be of clinical significance. In the next phase, we plan to conduct a multicenter study to establish an appropriate number of patients and to confirm efficacy by further evaluation including pathological assessment.

NAFLD/NASH is a disease with complex pathophysiology, and current therapies mostly target the pathogenesis and antimetabolic and anti-inflammatory components of the antifibrotic pathway. We, thus, propose a shift in focus to targeting Helz2 as a novel mechanism in our treatment.[21–23] This clinical trial is a phase IIa study to confirm the POC. After confirming the efficacy and safety of guanabenz acetate treatment in this study, we plan to discuss possible targets and staging in the next phase.

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Competing interests ANakajima reports grants and research support from Gilead, Mylan EPD, EA Pharma, Kowa, Taisho, and Biofermin. ANakajima is a consulting adviser for Gilead, Boehringer Ingelheim, BMS, Kowa, Astellas, EA Pharma and Mylan EPD. The other authors declare no conflicts of interest.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

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REFERENCES

1 Eguchi Y, Hyogo H, Ono M, *et al*. Prevalence and associated metabolic factors of nonalcoholic fatty liver disease in the general population from 2009 to 2010 in Japan: a multicenter large retrospective study. *J Gastroenterol* 2012;47:586–95.
2 Day CP. Natural history of NAFLD: remarkably benign in the absence of cirrhosis. *Gastroenterology* 2005;129:375–8.
3 Promrat K, Kleiner DE, Niemeier HM, *et al*. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* 2010;51:121–9.
4 Yokohama S, Yoneda M, Haneda M, *et al*. Therapeutic efficacy of an angiotensin II receptor antagonist in patients with nonalcoholic steatohepatitis. *Hepatology* 2004;40:1222–5.
5 Baum T, Shropshire AT. Studies on the centrally mediated hypotensive activity of guanabenz. *Eur J Pharmacol* 1976;37:31–44.
6 Yoshino S, Iwasaki Y, Matsumoto S, *et al*. Administration of small-molecule guanabenz acetate attenuates fatty liver and hyperglycemia associated with obesity. *Sci Rep* 2020;10:13671.
7 Imajo K, Kessoku T, Honda Y, *et al*. Magnetic resonance imaging more accurately classifies steatosis and fibrosis in patients with nonalcoholic fatty liver disease than transient elastography. *Gastroenterology* 2016;150:626–37.
8 Pokharel A, Kc S, Thapa P, *et al*. The effect of Empagliflozin on liver fat in type 2 diabetes mellitus patients with non-alcoholic fatty liver disease. *Cureus* 2021;13:e16687.
9 Eriksson JW, Lundkvist P, Jansson P-A, *et al*. Effects of dapagliflozin and n-3 carboxylic acids on non-alcoholic fatty liver disease in people with type 2 diabetes: a double-blind randomised placebo-controlled study. *Diabetologia* 2018;61:1923–34.
10 Loomba R, Kayali Z, Noureddin M, *et al*. GS-0976 reduces hepatic steatosis and fibrosis markers in patients with nonalcoholic fatty liver disease. *Gastroenterology* 2018;155:1463–73.
11 Harrison SA, Bashir MR, Guy CD, *et al*. Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2019;394:2012–24.
12 Patel J, Bettencourt R, Cui J, *et al*. Association of noninvasive quantitative decline in liver fat content on MRI with histologic response in nonalcoholic steatohepatitis. *Therap Adv Gastroenterol* 2016;9:692–701.
13 Simon TG, Roelstraete B, Khalili H, *et al*. Mortality in biopsy-confirmed nonalcoholic fatty liver disease: results from a nationwide cohort. *Gut* 2021;70:1375–82.
14 Neuschwander-Tetri BA, Loomba R, Sanyal AJ, *et al*. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015;385:956–65.
15 Sanyal AJ, Brunt EM, Kleiner DE, *et al*. Endpoints and clinical trial design for nonalcoholic steatohepatitis. *Hepatology* 2011;54:344–53.
16 Cadranel JF. [Good clinical practice guidelines for fine needle aspiration biopsy of the liver: past, present and future]. *Gastroenterol Clin Biol* 2002;26:823–4.
17 Ratziu V, Charlotte F, Heurtier A, *et al*. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005;128:1898–906.
18 Dulai PS, Sirlin CB, Loomba R. MRI and MRE for non-invasive quantitative assessment of hepatic steatosis and fibrosis in NAFLD and NASH: clinical trials to clinical practice. *J Hepatol* 2016;65:1006–16.
19 Costa-Silva L, Ferolla SM, Lima AS, *et al*. MR elastography is effective for the non-invasive evaluation of fibrosis and necroinflammatory activity in patients with nonalcoholic fatty liver disease. *Eur J Radiol* 2018;98:82–9.
20 Reeder SB, Robson PM, Yu H, *et al*. Quantification of hepatic steatosis with MRI: the effects of accurate fat spectral modeling. *J Magn Reson Imaging* 2009;29:1332–9.
21 Konerman MA, Jones JC, Harrison SA. Pharmacotherapy for NASH: current and emerging. *J Hepatol* 2018;68:362–75.
22 Sanyal A, Charles ED, Neuschwander-Tetri BA, *et al*. Pegbelfermin (BMS-986036), a PEGylated fibroblast growth factor 21 analogue, in patients with non-alcoholic steatohepatitis: a randomised, double-blind, placebo-controlled, phase 2A trial. *Lancet* 2019;392:2705–17.
23 Harrison SA, Rinella ME, Abdelmalek MF, *et al*. NGM282 for treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2018;391:1174–85.