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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.

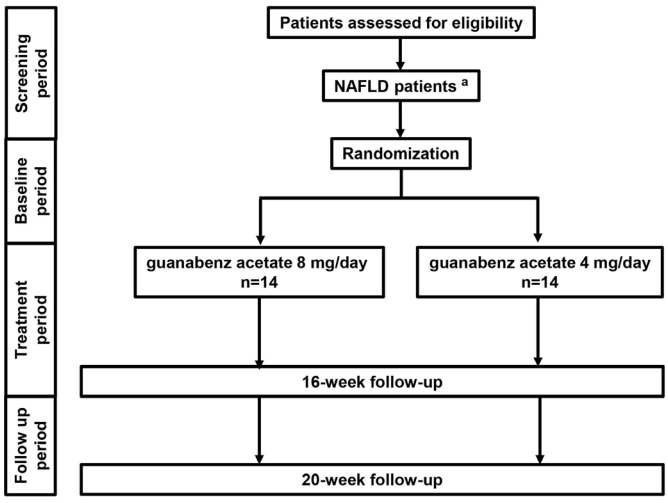


**Figure 1** Study design. [a]N=28 enrolled. NAFLD, non-alcoholic fatty liver disease.

### 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:<br>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<br>2. Liver fat content measured using MRI-PDFF<br>3. ALT, AST and γ-GTP<br>4. Weight<br>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)<br>6. HOMA-IR<br>7. Liver hardness (MRE)<br>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:<br>1. ALT>430 IU/L (males) or >240 IU/L (female); or AST>300 IU/L (males and females)<br>2. PT-INR≥1.5 (excluding anticoagulant therapy)<br>3. Total bilirubin value>2.0 mg/dL (excluding definitive diagnosis of Gilbert syndrome)<br>4. Platelet count<$8.0\times10^4$/μL<br>5. eGFR<45 (calculated by body surface area correction: standardised eGFR) |
| 4 | Patients diagnosed with NAFLD/NASH who meet criteria (1) or (2):<br>1. Patients diagnosed with NAFLD who meet the three criteria:<br>1. Diagnostic imaging or histological evidence of fatty liver<br>2. Alcohol intake<30 g/day for men and <20 g/day for women for ≥12 consecutive weeks 1 year before screening<br>3. Absence of other factors that cause fattening or chronic liver disease<br>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:<br>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<br>2. Patients with autoimmune hepatitis<br>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<br>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:<br>1. Insulin, GLP-1 receptor agonists, SGLT2 inhibitors or thiazolidine 12 weeks before screening<br>2. Ursodeoxycholic acid or vitamin E 12 weeks before screening<br>3. Dyslipidaemia or antihypertensive drugs whose doses were changed 12 weeks before screening<br>4. Oral diabetes treatment drug (DPP-4 inhibitor, SU preparation, α-glucosidase inhibitor, metformin) whose doses were changed 12 weeks before screening<br>5. Drugs known to have a significant effect on body weight (including over-the-counter drugs for weight loss) 12 weeks before screening<br>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<br>ALT<br>Alkaline phosphatase<br>Amylase<br>AST<br>Blood urea nitrogen<br>Chlorine<br>Creatinine<br>Estimated glomerular filtration rate* (during screening)<br>γ-GTP<br>Lactate dehydrogenase<br>Potassium<br>Sodium<br>Calcium<br>Total bilirubin<br>Total protein<br>Uric acid | HDL-C<br>LDL-C<br>Non-HDL-C*<br>TC<br>TG<br>Glucose<br>HbA1c<br>Insulin<br>HOMA-IR* | (Inflammation)<br>High-sensitivity C reactive protein<br>Ferritin<br>TNF-a<br>Interleukin 6<br>CK18/M30<br>Endotoxin LBP<br>Endotoxin activity<br>(Endocrine)<br>C-peptide<br>Total GLP-1/active GLP-1<br>Leptin<br>Adiponectin<br>(Fibrosis) hyaluronic acid<br>PIIIP<br>TIMP-1<br>M2BPGi<br>Type 4 collagen 7 s<br>ELF Score 3<br>Fibrosis-4*<br>(Fat)<br>Chylomicron cholesterol<br>Chylomicron triglyceride<br>Lipoprotein cholesterol<br>LDL triglyceride<br>VLDL cholesterol<br>VLDL triglyceride<br>Free cholesterol<br>Apolipoprotein A1<br>Apolipoprotein B<br>Adipsin<br>Free fatty acid<br>(Others)<br>TMAO |
| Haematological examination/coagulation (During screening, V2, V7, follow-up, termination) | Urinalysis (Screening, V2, V7, termination, follow-up) | |
| Haematocrit<br>Haemoglobin<br>Platelet count<br>Number of red blood cells<br>White blood cell count and white blood cell fraction (neutrophils, eosinophils, basophils, lymphocytes, monocytes)<br>International normalised ratio | Latent blood<br>Urine sugar pH<br>Urine protein<br>Specific gravity<br>Urobilinogen<br>Pregnancy test†<br>(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<br>TM6SF2 | Free thyroxine (FT4)<br>Free triiodothyronine (FT3)<br>Thyroid stimulating hormone | HBs antigen<br>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, $\theta$ 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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**Contributors** TKessoku and ANakajima will participate in the study. MI, TKessoku and TKobayashi will conduct a feasibility phase work. Recruitment of participants and follow-up will be performed by TKessoku, MI, KTanaka, AO, YK, AY, KTakahashi, TKobayashi, ANogami, YH, MY and SS. MRI reading will be performed by KI. MI, TKessoku, YH, YO, NK and ANakajima will participate in data interpretation. All authors will contribute to writing, reading and approving the final manuscript.

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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.

**Patient consent for publication** Not applicable.

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Clinical Trial Protocol Number：YCU - 21001
Ver1.1
Date Created：August 19th, 2021

# Informed Consent Form

## Phase II physician-initiated clinical trial investigating the efficacy and safety of guanabenz acetate for non-alcoholic fatty liver disease associated with hypertension
(G-Flash study)

Clinical Trial Protocol Number : YCU - 21001
Ver1.1
Date Created : August 19th, 2021

# table of contents

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# 1. Introduction

I will now explain to you about the clinical trial for your drug candidate, WY-8678 (guanabenz acetate). Please decide whether or not to participate in this clinical trial after fully understanding the contents of the explanation. You may take this explanation home with you and discuss it with your family members before making a decision. This is not mandatory. You are free to decide whether or not to participate in this clinical trial. Even after you have agreed to participate in the clinical trial, you may stop at any time, regardless of the reason. You will not be treated unfavorably or lose any medical benefits if you do not participate in the clinical trial, withdraw your consent during the clinical trial, or discontinue the clinical trial midstream.
If you have any questions or concerns, or need further explanation, please do not hesitate to ask your physician.

# 2. What is a Clinical Trial

In order for a new medication to be used by patients, its efficacy (effectiveness) and undesirable effects (side effects), etc., must be confirmed by the Ministry of Health, Labor and Welfare. To do this, we first look for ″drug candidate″ ingredients as shown in Figure 1-1 (Step 1). Then, we confirm in animals or other animals how the ingredient works (Step 2). Next, after confirming the safety of the drug in healthy volunteers, we investigate the ″effects″ and ″side effects″ of the drug in patients (Step 3). This type of study to investigate the drug as a drug is called a ″clinical trial″ (Chiken).

(Chiken). The drug candidate used in the study is called an ″investigational new drug. Clinical trials are conducted in consideration of the human rights and safety of the participants. The clinical trial is conducted in accordance with the ″Good Clinical Practice (GCP) for Drug Trials″ established by the Japanese government, taking into consideration the human rights and safety of

the participants. The results of the clinical trial, in which many patients cooperate, are compiled and finally reviewed by the government (Ministry of Health, Labour and Welfare) for approval as a new ″medicine″ (Step 4), and if approved, the drug will be used in the world (Step 5). If the drug is approved, it can be used in the market (Step 5). All the medicines we use today have had their efficacy and side effects confirmed through clinical trials.

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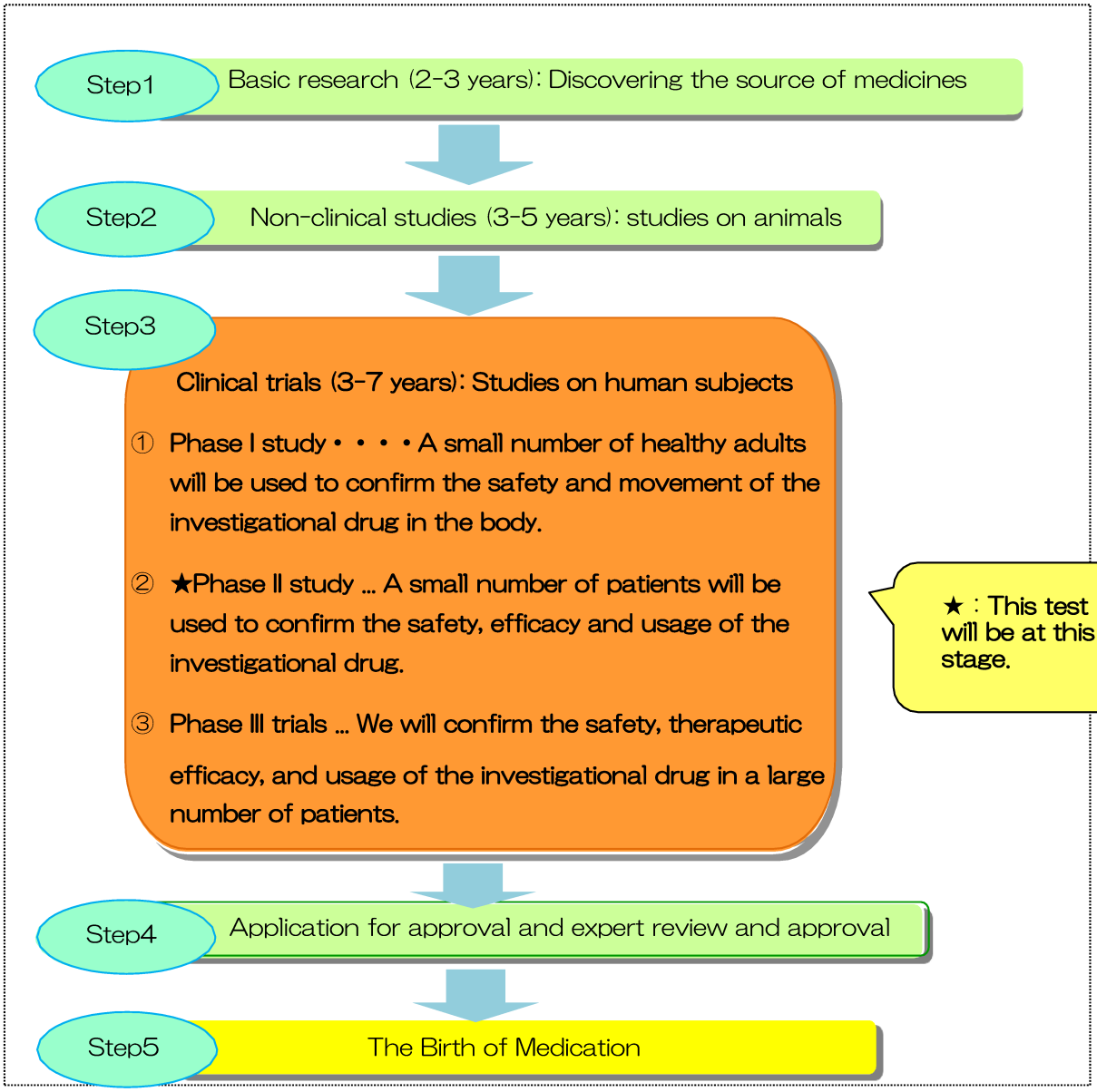
Figure 1-1 Flow chart of how a new drug is created



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In addition, clinical trials include the research aspect of obtaining new and unknown information as well as the objective of obtaining information necessary for drug review. Therefore, clinical trials are conducted in compliance with the strict rules and standards set by the government, and the content of the clinical trial plan is thoroughly reviewed and approved by the Clinical Trial Review Committee to ensure that it is scientifically and ethically valid and that there are no problems with its implementation. In addition, please understand in advance if you wish to participate in the clinical trial that the number of hospital visits and examinations may increase from usual.

## About the Clinical Trial Review Committee

Our hospital has a Clinical Trial Review Committee established by the hospital director, which includes physicians, non-physicians, and members of the public outside the hospital. This committee reviews and approves clinical trials to ensure that there are no scientific or ethical problems, and that the physicians involved in the trials are qualified. The committee also reviews whether the continuation of the clinical trial is appropriate.

The Clinical Trial Review Committee's procedure manual and review details can be found on the website below or at the Clinical Trial Management Office. For more information, please do not hesitate to contact your physician or the consultation desk.

< Our Clinical Trial Review Committee>

Establisher: Yokohama City University Hospital Hospital Director
Name: Clinical Trial Review Committee of Yokohama City University Hospital Type: Clinical Trial Review Committee of the Implementing Medical Institution
Location: 3-9 Fukuura, Kanazawa-ku, Yokohama City, Kanagawa Prefecture Website address: http://www-user.yokohamacu.ac.jp/~ynext/trial/irb/

## 3. About Your Disease

Your disease is called non-alcoholic fatty liver disease (shibokan shikan), a disease in which fat accumulates in the liver even though you do not drink much alcohol and may be caused by overeating, being overweight, or lack of exercise. (This type is not caused by excessive alcohol consumption.) These are divided into two types: ″nonalcoholic fatty liver,″ which progresses slowly, and ″nonalcoholic steatohepatitis,″ in which inflammation is added to nonalcoholic fatty liver, which progresses quickly, and some people may develop cirrhosis or even liver cancer if the disease gets worse.

## 4. About the investigational drug

WY- 8678 (guanabenz acetate), the investigational drug that will be used if you agree to participate in this clinical trial, has been approved for the treatment of essential hypertension since 1985. This investigational drug is believed to improve fatty liver by improving insulin resistance. Insulin resistance is a condition in which insulin, which controls blood sugar, does not work sufficiently due to obesity. It is expected to improve obesity, diabetes, and other conditions said to be related to nonalcoholic fatty liver disease.
The investigational drug in this study is a medication containing the active ingredient of guanabenz acetate.

## 5. Purpose of the Clinical Trial

The purpose of this clinical trial is to investigate the safety and efficacy of the investigational drug in patients with nonalcoholic fatty liver disease/nonalcoholic steatohepatitis complicated with hypertension by taking the investigational drug twice a day.

Specifically, the investigational drug containing 2 mg of guanabenz acetate will be evaluated when the patients take ″1 tablet twice a day″ and when they take ″2 tablets twice a day″. The efficacy and safety of the investigational drug containing 2 mg of guanabenz acetate will be studied when taken twice daily.

For details of the dosage method, please refer to ″(3) Usage of the investigational drug″ in ″6. Methods of the clinical trial″.

This clinical trial will be conducted only at our hospital, and approximately 28 people are expected to participate.

This clinical trial is being conducted with the support of Asuka Pharmaceuticals, Inc. for the costs related to its operation. However, we will not change your treatment policy or compromise the fairness of the clinical trial by giving priority to the interests of ASKA Pharmaceuticals Co.

## 6. Methods of the clinical trial

### Conditions for Participation in Clinical Trials

#### （1） Criteria for participation in the clinical trial

To ensure the safety of the clinical trial, the following conditions must be met in order to participate in this clinical trial. You will be asked to undergo several other tests, and the physician in charge will decide whether or not you can participate in the clinical trial. Depending on the results, you may not be able to participate in the clinical trial.

##### 1）Who can participate

- Patients between 20 and 75 years of age at the time of consent
- Patients who have given written consent in person

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- Patients who have been diagnosed with essential hypertension and whose blood pressure at screening is 130 mmHg or higher systolic and/or 85 mmHg or higher diastolic.
- Patients diagnosed with non-alcoholic fatty liver disease/non-alcoholic steatohepatitis
- Liver fat content of more than 8% on MRI-PDFF at screening
- MR elastography value less than 3.6 kPa at screening
- BMI greater than 25 kg/m2 at screening
- Patients who have been on diet and exercise therapy for 12 weeks prior to screening with no improvement
- Willingness to maintain a stable diet and physical activity throughout the study period

2) Who cannot participate?

- Pregnant, lactating, or possibly currently pregnant, or who do not agree to use contraception while participating in the clinical trial
- Have taken guanabenz acetate within 16 weeks prior to screening or have participated in other clinical studies (excluding observational studies)
- Have a drug allergy to guanabenz acetate
-
- Patients with hepatic insufficiency or cirrhosis
- Patients with the following laboratory findings ::

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1) ALT＞430 IU/L（males）ALT＞240 IU/L（females）or AST ＞300IU/L

2) PT-INR（prothrombin time - international normalized ratio）≧1.5 （excluding anticoagulation therapy）

3) Total bilirubin level > 2.0 mg/dl (except for confirmed diagnosis of Gilbert's syndrome)

4) Platelet count＜80,000／μL

5) eGFR＜45

- Patients with a history of acute or chronic liver disease or complications other than nonalcoholic fatty liver disease/nonalcoholic steatohepatitis

- Patients with a history of HIV infection

- Patients with symptoms of portal hypertension (complications: ascites, hepatic encephalopathy, varices, splenomegaly)

- Patients with a history of use of drugs related to non-alcoholic fatty liver disease or other hepatotoxins for more than 4 weeks in the year prior to screening以下の薬剤の使用がある方

1) Use of insulin, GLP-1 agonists, SGLT2 inhibitors, or thiazolidinediones in the 12 weeks prior to screening

2) Use of ursodeoxycholic acid or vitamin E in the 12 weeks prior to screening

3) Dose modification of dyslipidemia or hypertension medications in the 12 weeks prior to screening

4) Patients who have undergone dose modification of oral

diabetes medications (DPP-4 inhibitors, SU preparations, alpha-glucosidase inhibitors, metformin) in the 12 weeks prior to screening

5) Use of drugs known to have a significant effect on body weight (including over-the-counter drugs for weight loss) in the 12 weeks prior to screening

- Central nervous system depressants (barbital, thiopental sodium, morphine salt hydrate, flotillas, thiopental sodium, morphine hydrate, flotizolam, diazepam, etc.)
- Patients with a weight change of 10% in the 24 weeks prior to screening
- Patients who have undergone bariatric surgery or are scheduled to undergo surgery during the study period
- Patients with a history of type 1 diabetes mellitus
- Hemoglobin A1c (HbA1c) > 9.5% at screening or have poorly controlled type 2 diabetes mellitus
- Complicated hyperthyroidism or hypothyroidism, or those with screening results indicating thyroid dysfunction Except for those who received thyroid replacement therapy for hypothyroidism in the 12 weeks prior to screening and have stable laboratory values
- History of New York Heart Association class III or IV heart failure due to factors other than hypertension or IV heart failure due to factors other than hypertension
- History of myocardial infarction, unstable angina, percutaneous coronary intervention, coronary artery bypass graft, or stroke or

major surgical procedure in the 24 weeks prior to screening

- Patients with a history of drug abuse
- Patients with a malignant complication, except for those who have undergone radical surgery, completed chemotherapy/radiotherapy, or are undergoing hormone therapy.
- Patients already known to be intolerant to MRI examinations, or those for whom MRI examinations are contraindicated.
- Other patients whom the investigators deem inappropriate to conduct this clinical trial.

（2） Duration of Clinical Trial Participation

If you participate in this clinical trial, your participation period will consist of a screening period of up to 8 weeks, an investigational drug taking period of 16 weeks, and a follow-up period of 4 weeks, for a total of 28 weeks (maximum of approximately 7 months). If you decide to discontinue during the investigational drug taking period, you will still participate in the follow-up period (4 weeks after discontinuation) in principle.

（3） Usage of the investigational drug

If you are confirmed to meet the ″(1) Criteria for Participation in the Clinical Trial″ prior to the start of the clinical trial, you will be placed in one of the following dosing groups and will take the investigational drug for 16 weeks. You will be randomly assigned to one of the following groups in a 1:1 ratio according to a predetermined method, and neither you nor your physician will be able to choose which group you will be placed in. This is to remove any preconceived notions and to properly evaluate the efficacy and safety of the investigational drug.

【Dosage of the investigational drug】

| | |
|---|---|
| Group 1 | Study drug containing 2 mg of guanabenz acetate<br>1 tablet twice a day (2 tablets/day) |
| Group 2 | Study drug containing 2 mg of guanabenz acetate<br>2 tablets twice daily (2 tablets per day) |

• The investigational drug will be started after dinner on the first day of administration and will be taken until after breakfast on the day of the visit 16 weeks later.

• The investigational drug will be taken twice a day after breakfast and dinner. Guanabenz acetate should be taken one or two

tablets at a time.

• If you miss a dose, please take it at least 6 hours before the next dose.

• Store investigational drugs away from high temperatures and moisture.

（4） Schedule

After you give your consent to participate in this clinical trial, the examinations and observations listed in Table 6-1 will be performed according to the schedule. In addition, changes in physical condition or physician's orders may require visits other than those stipulated, additional tests may be required, or the amount of blood drawn may increase or decrease. The specific schedule is as follows

1）Screening

If you agree to participate in this clinical trial, you will undergo a screening to confirm that your current physical condition meets the criteria for participation in this clinical trial.

2）Treatment period

Patients who have been screened and found to be safe to participate in this clinical trial will be given the investigational drug. During the period of taking the investigational drug, we will conduct predetermined tests and observe the patient's physical condition and the progress of the disease to determine whether the patient can continue in the clinical trial.

3）Follow-up

Four weeks after completion of the study drug, the patient will continue to receive medical examinations and tests to check the patient's condition

4）Discontinuation

If the investigational drug is discontinued during the study period, the patient will undergo a medical examination and tests at the time of discontinuation. In addition, as a general rule, patients will be asked to come back to the clinic 4 weeks after the discontinuation visit to have a medical examination and tests.

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## Table 6-1 Clinical Trial Schedule

| | Consent acquisition | Screening | Treatment period | | | | | | Follow-up |
|---|---|---|---|---|---|---|---|---|---|
| | | V1 | V2 | V3 | V4 | V5 | V6 | V7／EOT | V8 |
| Week | | Within 8 weeks prior to registration | Prior to start of administration | 2 weeks | 4 weeks | 8 weeks | 12 weeks | 16 weeks | 4 weeks after end of administration |
| Tolerable range | | -8 weeks | - | ±3 days | ±7 days | ±7 days | ±7 days | ±7 days | ±7 days |
| Consent acquisition | ○ | | | | | | | | |
| Selection criteria | | ○ | ○ | | | | | | |
| Subject background | | ○ | | | | | | | |
| Serological test [a] | | ○ | | | | | | | |
| Chest X-ray | | ○ | | | | | | | |
| electro-cardiogram | | ○ | | | | | | | |
| Physical examination [b] | | ○ | ○ | | | | | ○ | |
| Vital signs c | | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Subjective and objective symptoms | | | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Pregnancy test [d] | | | ○ | | | | | ○ | |
| MRI[e] | | ○ | | | | | | ○ | |
| Liver biopsy | | △ | | | | | | | |
| Randomization | | | ○ | | | | | | |
| Hematology test / urine test [f] | | ○ | ○[j] | | | | | ○ | ○ |
| Endocrinological examination | | ○ | | | | | | | |
| Biochemical test 1 | | ○ | ○[j] | | ○ | ○ | ○ | ○ | ○ |
| Biochemical test 2[g] | | ○ | ○[j] | | | | | ○ | |
| Other [h] | | | ○ | | | | | ○ | |
| Somatic cell genetic test | | | ● | | | | | | |
| Providing drugs | | | ○ | | ○ | ○ | ○ | | |
| Checking the medication status | | | | ○ | ○ | ○ | ○ | ○ | |
| Survey of combination drugs | | ○ | ○ | ○ | ○ | ○ | ○ | ○ | |
| Investigation of adverse events | | | | ○ | ○ | ○ | ○ | ○ | ○ |

1) Height will be measured only at screening.

2) Only applicable female patients will have a urine pregnancy test performed prior to enrollment and at 16 weeks/discontinuation.

3) Liver biopsy results (within 32 weeks prior to screening) will be collected if available.

4) Blood volume: The maximum volume of blood to be collected is approximately 45 mL at one time, and approximately 140 mL of blood will be collected for the entire trial.

5) The collected blood samples will be stored for a maximum of 5 years, after which they will be disposed of in an appropriate manner. If the blood samples and the information obtained in this study are to be used in a newly planned research study for a purpose other than this clinical trial, we will do so only after obtaining approval from an ethics review committee, etc., which will deliberate the conduct of the research from an independent and fair perspective. In such cases, we will obtain your consent again before using the information.

6) MRI will be performed to measure MR elastography and MRI-PDFF; if the study is discontinued before 16 weeks, MRI will be performed at the end of treatment if you have completed at least 4 weeks of treatment.

7) Somatic cell genetic testing will be mandatory.

*If data is available within 1 month, it will be substituted and no new test will be performed.

（5） Somatic cell gene test

The somatic cell gene test will examine the types of genes, PNPLA3 and TM6SF2, which are known to be associated with non-alcoholic fatty liver disease/non-alcoholic steatohepatitis. These genes are not inherited by offspring due to genetic changes that occur at various stages of life. It is also thought to affect non-alcoholic fatty liver disease/non-alcoholic steatohepatitis, but since it has not been established as a diagnostic tool for the disease, the results of this test will not be shared with you.This test is mandatory for participation in this clinical trial and will require the collection of approximately 5 mL of blood. The blood sample will be stored for up to 5 years and then disposed of in an appropriate manner. If the blood samples and information obtained in this study are to be used in a newly planned research study for a purpose other than this clinical trial, it will be done only after approval is obtained from an ethics review committee that deliberates on the conduct of the research from an independent and fair standpoint. In such cases, we will obtain your consent again before using the information.

## 7. Expected effects and side effects

＊Expected effects

It is expected to improve non-alcoholic fatty liver disease by improving the inadequate insulin action secreted by the pancreas.

＊Expected inconvenience

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In this clinical trial, patients will be assigned to either one or two doses of the investigational drug containing 2 mg of guanabenz acetate per dose, but not all patients in either group may benefit. In addition, clinical trials may require a greater number of office visits and tests than in the general population, and the medical examinations may take longer. There are other medications and treatments that should not be used while participating in a clinical trial. If you wish to use any of the medications or treatments listed below while participating in a clinical trial, please consult your physician in advance.

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【Medications that should not be used during the clinical trial】

| Drugs | Period |
|---|---|
| (1) Ursodeoxycholic acid | From the time consent is obtained to the end of the treatment period |
| (2) Guanabenz analogs (clonidine, methyldopa) | |
| (3) Thiazolidine, glucagon-like peptide-1 (GLP-1) receptor agonist, SGLT2 inhibitor, insulin | |
| (4) Central nervous system depressants (barbital, sodium thiopental, morphine hydrochloride hydrate, brotizolam, diazepam) | |
| (5) Vitamin E | |
| (6) NAFLD-related drugs (amiodarone, methotrexate, systemic glucocorticoids, tetracycline, tamoxifen, higher doses of estrogen, anabolic steroids, or valproic acid than used for hormone replacement) or other hepatotoxins | |
| (7) Drugs that significantly affect body weight (including over-the-counter weight loss drugs) | |

【From the time of screening to the follow-up end, the following therapies are prohibited:】

| Therapy | Period |
|---|---|
| (1) Obesity surgery (sleeve gastrectomy, gastric bypass surgery, sleeve bypass surgery, etc.) | From the time of screening to the end of follow-up |

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【Drugs restricted for concomitant use include】

| Drugs | Period |
|---|---|
| (1) Antihypertensive drug | During the treatment period |
| (2) Drugs to treat dyslipidemia | |
| (3) Drugs to treat diabetes treatment (DPP-4 inhibitor, SU preparation, α-glucosidase inhibitor, metformin) | |

＊About Side Effects

Guanabenz acetate is already marketed in Japan as a medication to improve essential hypertension, and the following side effects have been reported so far. Therefore, similar side effects may occur.

Not all of these side effects will occur in all patients. On the other hand, there is a possibility that unexpected side effects other than those listed here may occur, and it cannot be denied that some side effects may be serious and life-threatening.

Safety information on guanabenz acetate (product name: Wytens Tablets 2 mg) marketed in JapanSafety information of guanabenz acetate (product name: Wytens Tablets 2 mg) marketed in Japan. In 822 out of 15,358 cases (5.4%) investigated at the time of reexamination of guanabenz acetate by the Japanese Ministry of Health, Labour and Welfare (MHLW), adverse reactions were observed. The most common adverse reactions were gastrointestinal symptoms such as dry mouth (2.9%), neuropsychiatric symptoms such as drowsiness and dizziness (2.8%), and hypersensitivity symptoms such as rash (0.2%). There were no serious adverse reactions, but other adverse reactions that occurred were as follows by frequency. Please inform your doctor immediately if you experience any change in your physical condition or any symptoms that concern you in the slightest.

Appropriate treatment will be given. During the clinical trial, periodic examinations and consultations will be conducted to check for such undesirable symptoms. Unpredictable side effects may occur in addition to those listed here. Please ask your physician for the most up-to-date information regarding side effects at any time.

| | 0.1 ～0.5% | Less than 0.1% |
|---|---|---|
| Hypersensitivity | Rash | Facial eczema, urticaria, pruritus |
| Liver | — | AST (GOT), ALT (GPT) elevation ALT (GPT) increase |
| Neuropsychiatric systems | Drowsiness, dizziness, lightheadedness, dizziness, dizziness, fatigue, weakness, headache/overhead | Tinnitus, insomnia, depression, tremor |
| Circulatory System | — | palpitations, chest pain, bradycardia, arrhythmia, excessive hypotension |
| Digestive Organs | Dry mouth, abdominal discomfort, nausea | anorexia, diarrhea, constipation, vomiting, heartburn, bitter taste, stomach pain |
| Other | — | stiff shoulders, back pain, myalgia, numbness, cold extremities, nasal obstruction, dyspnea, facial flushing, edema, bladder tenesmus, urinary frequency Bladder tenesmus, Frequent urination |

## 8. What to do when new information regarding the clinical trial is obtained

If there are any changes to the plan or other aspects of this clinical trial, we will explain those changes to you. If we obtain any information that may affect your willingness to participate in the clinical trial, such as information on new side effects obtained during your participation in the clinical trial, we will promptly inform you of the details of such information. At that time, we will ask you again if you are willing to continue to participate in this clinical trial. However, as described in ″9. Discontinuation of the clinical trial,″ you may withdraw from the clinical trial at any time, as your participation in the clinical trial is of your own free will.

## 9. Discontinuation of the clinical trial

Even after you have participated in this clinical trial, the trial may be terminated in the following cases.

（1） When this clinical trial is discontinued

1) When there are unavoidable ethical or medical reasons to ensure patient safety

2) If the scientific validity of the development of this drug is lost

3) If the investigator or the investigational site violates any ministerial ordinance, clinical trial plan, or various procedures that should be observed by the investigator or the investigational site, which is recognized as an obstacle to the proper conduct of the clinical trial.

4) When the investigator decides to discontinue or suspend the clinical trial

5) When the investigator is instructed by the investigational review committee to discontinue the clinical trial

（2） When your participation in a clinical trial is terminated

1） If you request discontinuation of the clinical trial

2) If the results of tests or your symptoms are found not to meet the conditions for participation in the clinical trial

3) If you take any medication that should not be used during the clinical trial period or receive any treatment that is prohibited

4) If your physical condition is so poor that it is difficult for you to continue the clinical trial

5) If the physician in charge decides that it is better to discontinue the study in any other case. If you discontinue the study after starting to take the investigational drug, please cooperate with the tests marked with a circle in the ″16 weeks/at discontinuation″ column of the schedule chart shown in Table 6-1 in ″6.

You will not be disadvantaged in any way in subsequent treatment after discontinuation of the clinical trial. Your treatment for your disease will be the most appropriate treatment for you from among the usual treatments. Please note that even if the clinical trial is terminated, we may use the results up to that point.

## 10. About other treatment methods

Currently, there are no drugs for nonalcoholic fatty liver disease that are covered by insurance in Japan, and the treatment method most often used is to improve lifestyle through diet and exercise therapy. This may improve obesity, diabetes, dyslipidemia, hypertension, and other conditions that underlie the disease, and may also lead to improvement of nonalcoholic fatty liver disease. If lifestyle modifications do not show sufficient benefit, the disease may be treated indirectly with medications for hypertension, dyslipidemia, and diabetes, but it is not yet clear whether these will have long-term benefits. If you do not participate in this clinical trial, or even if you stop the trial midway through, you can still consult with your

physician to choose an appropriate treatment option that is tailored to each patient's individual situation.

## 11. In case of adverse health effects

If you experience unusual symptoms while participating in a clinical trial, please contact your physician immediately. Appropriate care and treatment will be given immediately.

If you experience any side effects or other health problems due to your participation in this clinical trial during or after the trial is completed, your physician will provide you with the best possible treatment. You may also be entitled to compensation for such health problems. However, you may not receive compensation or your compensation may be limited if you did not follow the instructions of your physician, if your health damage was caused by your negligence or intention, or if it becomes clear that your health damage was not related to this clinical trial.

Please keep the receipt issued by the medical institution in a safe place, as it will be necessary for you to receive compensation.

For details on compensation, please refer to the attached document (″Compensation for Health Damage Caused″).

## 12. Reduction of costs associated with participation in a clinical trial

There is no cost for the investigational drug during this clinical trial. The investigational drug will be provided free of charge. However, you will be asked to pay the insurance portion of the cost of the tests (blood tests, MRI scans, etc.) performed at our hospital and the cost of medications other than the investigational drug.

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In order to reduce your transportation costs, we will pay 10,000 yen per visit for the clinical trial as a burden reduction fee. The maximum amount of payment is 80,000 yen depending on the number of times you visit the clinic. This will be transferred to your designated account by ″Yokohama Shiritsu Daigaku″ in a lump sum after the completion of the clinical trial period. Please read the separate document explaining the receipt of the Clinical Trial Cooperation Fee. This burden reduction fee may be declined for any reason.

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## 13. About personal information

In order to confirm that this clinical trial has been conducted correctly, including in cases where you withdrew your consent in the middle of the trial, the people involved in the clinical trial may inspect your medical records (e.g., medical records) related to the trial. The people involved in the clinical trial include personnel from the company commissioned by the investigator, people from the Clinical Trial Review Committee, people from the Ministry of Health, Labor and Welfare, and officials commissioned by the government to conduct the investigation. These people are required by law not to leak the contents of medical records and other records to outside parties, and information about your privacy will not be leaked outside the hospital.

The results obtained from this clinical trial will be reported to the company that is providing the costs related to the operation of this clinical trial, and may be used as part of the materials submitted to the government (Ministry of Health, Labor and Welfare). In addition, data obtained from this clinical trial may be presented in medical papers or at academic conferences. The information that will be used to identify you in the clinical trial report will not be your name, address, or other personal information, but rather an identification code that is a combination of numbers and letters. The list linking this identification code to your name and other information is maintained in the hospital and is controlled by the hospital's regulations on personal information management. However, among the personal information, information such as date of birth may be entered in the clinical trial report form for reasons such as to confirm the criteria for participation in the clinical trial, but even in this case, this information will not be leaked to the outside world or used for purposes other than this clinical trial. In handling personal information, we will give due consideration to the protection of your privacy. By signing the consent form after listening to this explanation, you agree to the access to your medical records and the use of your personal information (date of birth, etc.). By signing the consent form after listening to this explanation, you consent to the access to your medical records and the use of your personal information (date of birth, etc.)。

## 14. What you should follow

For your safety and to collect reliable data, please observe the following

(1) When you come to the clinic at 4, 8, 12, and 16 weeks/discontinuation, you will need to fast for 8 hours prior to the blood test. Therefore, please take the study drug at the following times.

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If you visit the clinic in the morning, please take the study drug without eating breakfast before coming to the clinic.
If you visit the clinic in the afternoon, please take the investigational drug before breakfast as usual and come to the clinic without eating lunch. If you visit the clinic in the afternoon, please take the study drug before breakfast as usual and visit the clinic without eating lunch.
(2) During your participation in the clinical trial, please maintain a stable diet and a normal lifestyle (physical activity).
(3) Please fill in the medication diary as instructed by the investigator or clinical trial coordinator to keep track of the medication you are taking and your daily condition.
(4) If you visit another hospital, please inform that physician that you are participating in a clinical trial. Also, please inform your physician or the clinical trial coordinator that you have seen another physician. With your permission, we may inquire with the physician at the other hospital about your medications and your condition.
(5) Please be sure to come to the hospital on your scheduled visit date. (If you are unable to make it, please let us know in advance.)
(6) If you are currently using any medications (including over-the-counter medicines and health foods), if you will be using any new medications after participating in the clinical trial, or if you will be receiving any new treatment in addition to the medications you are currently using, please contact your doctor in charge in advance. Medications may interact with each other, which means that they may have a negative effect on your health if used together, either by losing their effectiveness or by having a stronger effect.

(7) If you feel that something is wrong with your body, such as a different physical condition from usual (including broken bones, accidents, etc.), please contact your doctor anytime.
(8) On the day of your visit, please bring everything with you, including your medication log, extra study medication, study medication bag, and empty study medication sheets.
(9) Women of childbearing potential must use oral contraceptives, a contraceptive ring (IUD), pessary or condom, or other contraceptive methods during participation in the clinical trial. You will be asked to choose a contraceptive method that is appropriate for you in consultation with the investigator. If you believe that your contraceptive method was inadequate or if you become pregnant, please inform us immediately.
(10) Please be careful when engaging in hazardous activities such as drinking alcohol, working at high altitudes, driving a car, etc. while you are taking investigational drugs.
(11) Please inform your physician of any changes in your address, telephone number, or other contact information.

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# 15. Disaster Message Dial in the Event of a Large-Scale Disaster

In the event of a major disaster, we may contact you at the telephone numbers (including those of your family members) that we have confirmed in advance in order to confirm your safety. In the event that communication networks are disrupted, we ask for your cooperation in using the Disaster Message Dial (171). The Disaster Message Dial is a service set up 30 minutes after an earthquake of intensity 6 or higher on the Japanese seismic intensity scale to allow people in disaster-stricken areas to register and confirm their safety via telephone or the Internet.



Iwaki M, *et al. BMJ Open* 2022; 12:e060335. doi: 10.1136/bmjopen-2021-060335

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【How to record a message on the Disaster Message Dial ″171】

① Dial 171. Guidance will be given.

② Dial 1 (recording).

③ Dial your home phone numbe

④ You will be asked for the type of telephone (push type or dial type).

⑤ Follow the guidance and record your name and contact information.

【How to play Disaster Message Dial ″171】

① 1 Dial 171. Guidance will be given.

② Dial 2 (playback).

③ Dial the phone number of the person you wish to contact

④ You will be asked for the type of telephone.

⑥ You will be told from the new message.

【Disaster broadband message board ″web171】

① Search for Web171 and visit
https://www.web171.jp/

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## 16. Contact point for clinical trials

Yokohama City University Hospital

(1) Name of the investigator: Takaomi Shigurashi Name of your physician in charge:
Contact point for clinical trials: Yokohama City University Hospital
Contact point: 045-787-2800 (main number)

(2) Contact for consultation: Clinical Trial Management Office, Yokohama City University Hospital
Phone: 045-352-7 510 (Weekdays 9:00-17:0) 0)

(3) Contact on nights and holidays
Phone: 045-787-2 800 (Representative number)

*In case of emergency during nights and holidays, please contact the above and consult with the physician on duty in the Department of Gastroenterology.



Iwaki M, *et al. BMJ Open* 2022; 12:e060335. doi: 10.1136/bmjopen-2021-060335

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# Attachment 1 Explanation of Terms

| No. | Term | Explanation |
|---|---|---|
| 1) | Cirrhosis | This is a disease in which the liver cells become inflamed, and when the inflammation is repaired over and over again, the liver becomes hard and loses function. |
| 2) | Hepatic cancer | A disease in which liver cells become cancerous. |
| 3) | Essential hypertension | This is a type of hypertension with no known cause, and accounts for about 90% of all hypertension cases. Intrinsic hypertension is a lifestyle-related disease that is related to genetic factors and environmental factors such as lifestyle. It is said to be a lifestyle-related disease. |
| 4) | Insulin. | This hormone is essential for the efficient use of glucose. It is secreted by the pancreas. |
| 5) | MRI-PDFF | One type of test performed using MRI to measure the amount of fat in the liver. |
| 6) | MR Elastography | The liver is vibrated and the vibrating liver is MRI to image and measure the stiffness of the liver. |
| 7) | BMI | Body mass index, a value indicating body mass index, which is calculated based on the relationship between weight and height. |

| | | |
|---|---|---|
| 8) | Liver failure | A condition in which the liver function is greatly reduced and is unable to fulfill its role This is a condition in which the liver becomes. |

| No. | Term | Explanation |
|---|---|---|
| 9) | ALT | Abnormalities in the liver cause high blood levels, and it is one of the enzymes normally found in cells. |
| 10) | AST | Abnormalities in the liver, heart, or muscles cause blood levels is elevated and is one of the enzymes normally present in cells. |
| 11) | PT - INR | This test measures how long it takes for the blood to clot. |
| 12) | Bilirubin | It is a pigment formed when red blood cells break down. It is a pigment that is formed when red blood cells break down. A type of cellular component of the blood. |
| 13) | Platelet | A type of cellular component of blood. |
| 14) | e GFR | It indicates how well the kidneys are able to excrete waste products into urine. The lower the value, the worse the kidney function is. |
| 15) | HIV | The lower the value, the poorer the kidney function. A virus that infects a person's immune cells and causes acquired immunodeficiency syndrome (AIDS) |

| No. | Term | Explanation |
|---|---|---|
| 16) | Ascites | The membrane that surrounds the organs in the abdomen is called the peritoneum. The peritoneum creates a space called the peritoneal cavity to reduce friction between organs. The peritoneal cavity normally contains 20 to 50 mL of water, but if a greater volume of water than usual accumulates due to various diseases, the condition is called ascites. |
| 17) | Hepatic encephalopathy | This complication occurs when toxic substances (e.g., ammonia) that would be metabolized by the normal liver reach the brain. |
| 18) | Vascular aneurysm | The veins in the hands and feet have valves to prevent backflow and allow blood to return to the heart. When these valves fail to work properly, causing backflow, or when large veins become clogged, the venous pressure becomes high. For these reasons, the veins near the skin become large, long, and swollen. |
| 19) | Splenomegaly | This is a condition in which the spleen becomes swollen and enlarged. |
| 20) | Portal hypertension | Blood flow from the intestinal tract and spleen passes through the ″portal vein″ to the portal vein″ to the liver. Portal hypertension is a condition in which the portal hypertension is a condition in which |

| | | |
|---|---|---|
| | | the blood flow in the portal vein is blocked for some reason and the blood pressure in the portal vein rises. |

| No. | Term | Explanation |
|---|---|---|
| 21) | GLP-1 agonist | The drug binds to the GLP-1 receptor in the pancreas and stimulates the secretion of insulin, thereby lowering blood glucose levels. This is a drug that lowers blood glucose levels. |
| 22) | SGLT2 inhibitor | A drug that lowers blood glucose levels by suppressing the uptake of glucose from the renal tubules and facilitating the excretion of sugar in the urine. |
| 23) | DPP-4 inhibitor | A medication that lowers blood glucose levels by strengthening the function of the hormone incretin in the pancreas. |
| 24) | SU preparation | Sulfonylureas. A drug that stimulates insulin secretion in the pancreas. A medication that stimulates insulin secretion in the pancreas. |
| 25) | Alpha glucosidase inhibitor | By slowing down the digestion and absorption of sugar in the small intestine This is a drug that improves postprandial hyperglycemia by slowing down the digestion and absorption of sugar in the small intestine. |
| 26) | CNS depressants | A drug that acts on the central nervous system and suppresses its function. They include sedatives, tranquilizers, and sleep inducers. |

| | | |
|---|---|---|
| 27) | Beta-blockers | Medications that improve hypertension, angina pectoris, heart failure, etc. by suppressing blood pressure, heart rate, etc. |

| No. | Term | Explanation |
|---|---|---|
| 28) | Type 1 glycosuria Disease | Diabetes is a disease that causes blood glucose levels to rise due to poor insulin function, and is classified into several types depending on its cause. Type 1 diabetes occurs when the beta cells that make insulin in the pancreas are destroyed, resulting in a weakened ability to produce insulin or the inability to produce insulin. |
| 29) | HbA1c (Hemoglobin A1c) | Hemoglobin, a protein in the red blood cells of the blood (Hb), a protein bound to glucose. High blood glucose levels result in a high HbA1c. The HbA1c value obtained from a blood test can be used to estimate the state of blood glucose from that day to about two months ago. |
| 30) | Type 2 glycosuria | Blood glucose levels become high when insulin production becomes difficult (insulin hypoglycemia) or insulin becomes less effective (insulin resistance). In addition to genetic influences, there are environmental causes of type 2 diabetes, such as overeating, lack of exercise, and obesity. |

| No. | Term | Explanation |
|---|---|---|
| 31) | Hyperthyroidism | This is a disease in which the thyroid gland produces too much thyroid hormone and is overactive. Common symptoms include swelling of the thyroid gland, tachycardia (rapid pulse), trembling of the fingers, sweating easily, weight loss despite eating a lot, irritability, fatigue, and occasional weakness of the limbs. |
| 32) | Hypothyroidism | The thyroid hormone action in the blood is lower than necessary. Common symptoms of hypothyroidism include lethargy, fatigue, swelling, coldness, weight gain, slowness of movement, poor memory, and constipation. |
| 33) | Myocardial infarction | It is a condition in which the coronary arteries become completely clogged or thinned rapidly, causing the heart muscle cells to die and lose function. |
| 34) | Unstable angina pectoris | Angina attacks become more and more frequent. and occur not only with exertion, but also at rest. |
| 35) | Percutaneous coronary intervention | When a narrowed coronary artery is found, the narrowed coronary artery can be catheterized. The narrowed coronary artery is widened using a catheter to |

| | | |
|---|---|---|
| | | facilitate the flow of blood. |

| No. | Term | Explanation |
|---|---|---|
| 36) | Coronary artery bypass grafting | This surgery is performed for angina pectoris or myocardial infarction caused by narrowing or clogging of the coronary arteries. |
| 37) | Stroke | This is a disease in which the brain is damaged due to clogging or rupture of blood vessels in the brain. |
| 38) | MRI scan | An examination in which the patient is placed inside a cylinder made of powerful magnets and the organs and blood vessels of the body are photographed using the power of magnetism. |
| 39) | Liver biopsy | It is a test in which a part of the liver is removed to diagnose the disease. |
| 40) | Shaking of the liver | A ″tremor″ that occurs even though one does not intend to move. |
| 41) | Bladder tenesmus | A condition in which a strong urge to urinate occurs immediately after urination. |

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Attachment 2 **Compensation in the Event of Health Damage**

# Phase II physician-initiated clinical trial investigating the efficacy and safety of guanabenz acetate for non-alcoholic fatty liver disease associated with hypertension
(G-Flash study)

**（physician-initiated clinical trial）**

Although this clinical trial will be conducted with the utmost care, we have established policies and procedures regarding compensation in the unlikely event that you suffer any adverse health effects as a result of the investigational drug or the conduct of the clinical trial.
This document is intended to explain in more detail the indemnity provisions of the Consent Explanation Document. Please keep this document together with a copy of the consent document.
If you experience any side effects or other health problems, please do not hesitate to report them to your physician, clinical trial coordinator, etc. We will take appropriate measures that we believe are best for you, including treatment.

## 1.compensation for any health problems that may occur during this clinical trial

### (1) Principle of Compensation

1) Compensation is to appropriately compensate for the loss in the event of a health hazard to you, even if the medical institution is not legally responsible (even in the absence of

negligence), based on the purpose of the GCP ordinance (rules for conducting a clinical trial set by the government).

2) If your participation in this clinical trial results in any health problems, you will be compensated in accordance with the Compensation Policy and Procedures.

3) If you are found liable, you may file a lawsuit for damages. This compensation plan does not preclude you from exercising your right to claim damages.

## (2) Compensation standards

The compensation for this clinical trial includes disability compensation and survivors' compensation in the event of residual disability (Grade 1 or 2) or death. Medical expenses and medical benefits will not be paid in this clinical trial.
Furthermore, compensation in the form of medical care will be provided for health problems other than residual disability (Grade 1 to 2) and death. Medical expenses in such cases will be paid by your health insurance, and you will be asked to bear a portion of the medical expenses.

## (3) Cases not covered by the indemnity

1) If there is no causal relationship between your health damage and this clinical trial, you are not eligible for compensation.
2) If we or our investigators or other third parties are legally responsible for your health damage, you are not eligible for compensation.
3) Damage to your health caused by your willful act is not covered by the compensation.

## (4) When limiting coverage

If the health problem was caused by your gross negligence (e.g., making a false or false declaration, failing to follow the dosage and administration instructions given, or not following the instructions of the investigator), the compensation payment may be reduced or you may not receive compensation.

## 2. Compensation procedures

### (1) What to do in the event of a health hazard

If you suffer any health problems as a result of this clinical trial, we will take the necessary measures that we believe are best for you, including treatment.

### (2) Offers of Compensation

If you believe that you have suffered from side effects or other health problems, please notify your physician, clinical trial coordinator, or other relevant personnel. The investigator will determine the causal relationship between the adverse health effects and the clinical trial, and will explain to you whether the adverse health effects are compensable or not.
If you have any other questions regarding compensation, please do not hesitate to contact the investigator.

## 3. Handling of Personal Information

In accordance with the ″Personal Information Protection Law,″ your personal information that we obtain in the course of providing compensation will be appropriately managed and taken care of, and will not be used for any purpose other than the payment of compensation.

## 4. Other

If you have any questions regarding compensation, please do not hesitate to contact the investigator, clinical trial coordinator, or clinical trial consultation service as indicated in the explanatory document.

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# Consent form

Registration Number （___-_____） 【For medical records】

I have been informed of the details of this clinical trial (Phase II physician-initiated clinical trial investigating the efficacy and safety of guanabenz acetate for non-alcoholic fatty liver disease associated with hypertension), and I fully understand and agree to participate in this clinical trial on my own volition.

I have also received and retain a copy of the explanation document and this consent form.

Signature of the clinical trial participant

Date of agreement (YYYY/MM/DD)：_____/____/
Name：

Agree to receive burden reduction costs（ Yes • No ）

Signature of the physician who described the research

Date of explained (YYYY/MM/DD)：_____/____/
Name：

Signature of the collaborator who provided supplementary information

Date of explained (YYYY/MM/DD)：_____/____/
Name：

Date of consent received (YYYY/MM/DD)：_____/____/

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# Consent form

Registration Number （ - ） 【For Clinical Trial Office】

I have been informed of the details of this clinical trial (Phase II physician-initiated clinical trial investigating the efficacy and safety of guanabenz acetate for non-alcoholic fatty liver disease associated with hypertension), and I fully understand and agree to participate in this clinical trial on my own volition.

I have also received and retain a copy of the explanation document and this consent form.

Signature of the clinical trial participant

Date of agreement (YYYY/MM/DD)： / /
Name：

Agree to receive burden reduction costs（ Yes • No ）

Signature of the physician who described the research

Date of explained (YYYY/MM/DD)： / /
Name：

Signature of the collaborator who provided supplementary information

Date of explained (YYYY/MM/DD)： / /
Name：

Date of consent received (YYYY/MM/DD)： / /

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# Consent form

Registration Number （___-_____） 【For patient storage】

I have been informed of the details of this clinical trial (Phase II physician-initiated clinical trial investigating the efficacy and safety of guanabenz acetate for non-alcoholic fatty liver disease associated with hypertension), and I fully understand and agree to participate in this clinical trial on my own volition.

I have also received and retain a copy of the explanation document and this consent form.

Signature of the clinical trial participant

Date of agreement (YYYY/MM/DD)：_____/____/
Name：

Agree to receive burden reduction costs（ Yes • No ）

Signature of the physician who described the research

Date of explained (YYYY/MM/DD)：_____/____/
Name：

Signature of the collaborator who provided supplementary information

Date of explained (YYYY/MM/DD)：_____/____/
Name：

Date of consent received (YYYY/MM/DD)：_____/____/

clinical trial protocol 番号：YCU-21001

# Phase II physician-initiated clinical trial investigating the efficacy and safety of guanabenz acetate for non-alcoholic fatty liver disease associated with hypertension

(G-Flash study)

# Clinical trial protocol

Yokohama City University Hospital
Department of Palliative Medicine
Takaomi Kessoku

Ver 1.1

clinical trial protocol 番号：YCU-21001

Created: August 19, 2021

clinical trial protocol 番号：YCU-21001

Version history

| Version No. | Creation date |
|---|---|
| Ver 1.0 | July 16, 2021 |
| Ver 1.1 | August 19, 2021 |
| | |
| | |
| | |

Confidentiality

This clinical trial protocol is confidential information and is provided to the coordinating investigator, principal investigator, sub-investigator, clinical trial collaborators, investigative drug administrator, implementing medical institution, and institutional review board members participating in this clinical trial. This clinical trial protocol may not be disclosed to a third party or used for any purpose other than the purpose of this clinical trial without the written consent of Yokohama City University Hospital, except when explaining the contents of this clinical trial to the subjects.

Summary

| | |
|---|---|
| Clinical trial title | Phase II physician-initiated clinical trial investigating the efficacy and safety of guanabenz acetate for non-alcoholic fatty liver disease associated with hypertension (G-Flash study) |
| Investigational drug name | WY-8678 |
| Targeted adaptation | Non-alcoholic fatty liver disease/non-alcoholic steatohepatitis （NAFLD/NASH） |
| Clinical trial method, clinical trial design | Single-center, randomized |
| Clinical trial period | May 1, 2021 to June 30, 2023<br>(Consent acquisition period: September 1, 2021 to July 31, 2022) |
| clinical trial protocol Identification code | YCU-21001 |
| Development phase | Phase IIa |
| Purpose | To investigate the efficacy and safety of 4 mg/day of WY-8678 (guanabenz acetate) and 8 mg/day of WY-8678 (guanabenz acetate) in patients with non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH patients) with hypertension |
| Target number of subjects | Number of analyzed cases: 28 |
| Incorporation criteria | **Selection criteria**<br>1. Patients who have received a full explanation about this study and who have provided written consent.<br>2. Patients ≥ 20 years of age ≤ 75 years of age at the time consent was provided.<br>3. Patients diagnosed with essential hypertension and whose systolic blood pressure at the time of screening is ≥ 130 mmHg and/or diastolic blood pressure is ≥ 85 mmHg (according to the diagnostic criteria for metabolic syndrome)<br>4. Patients diagnosed with NAFLD/NASH who meet the following criteria (1) or (2)<br>(1) Patients diagnosed with NAFLD who meet the following three criteria:<br>① Diagnostic imaging or histological evidence of fatty liver,<br>② Alcohol intake < 30 g/day for men and < 20 g/day for women for 12 or more consecutive weeks one year before screening,<br>③ Absence of other factors that cause fattening or chronic liver disease.<br>(2) Patients with a definitive diagnosis of NASH by biopsy within 32 weeks before screening<br>* The definitive diagnostic criteria for NASH are defined as a fibrosis stage in liver biopsy in the evaluation using the "NASH Clinical Research Network (CRN) criteria" by an F1-F3 pathologist and a NAFLD activity score (NAS) ≥4 points (each item has one or more points):<br>① Fattening (0-3 points)<br>② Balloon-like swelling (0-2 points) |

| | |
|---|---|
| | ③ Inflammation in the lobules (0-3 points)<br>5. Patients with magnetic resonance imaging (MRI)-proton density fat fraction (PDFF) liver fat mass ≥ 8% at screening.<br>6. Patients with magnetic resonance elastography (MRE) value ≤ 3.6 kPa at screening.<br>7. Patients with a body mass index (BMI) ≥ 25 kg/m$^2$ at the time of screening.<br>8. Patients receiving diet or exercise therapy 12 weeks before screening, with no improvement.<br>9. Patients who are willing to maintain a stable diet and physical activity during the clinical trial.<br><br>**Exclusion criteria**<br>1. Pregnant, lactating, potentially pregnant women, or patients who do not agree to contraception during the trial period.<br>2. Patients who have taken guanabenz acetate within 16 weeks prior to screening or who have participated in other clinical studies (observational studies are excluded).<br><br>3. Patients with drug allergies to guanabenz acetate.<br>4. Patients with liver failure or cirrhosis.<br>5. Patients with the following laboratory test values:<br>(1) Alanine aminotransferase (ALT) > 430 IU/L (males) or > 240 IU/L (female); or aspartate aminotransferase (AST) > 300 IU/L (males and females)<br>(2) Prothrombin time-international normalized ratio (PT-INR) ≥ 1.5 (excluding anticoagulant therapy)<br>(3) Total bilirubin value > 2.0 mg/dL (excluding definitive diagnosis of Gilbert syndrome)<br>(4) Platelet count < 80,000/μL<br>(5) Estimated glomerular filtration ratio (eGFR) < 45 (calculated by body surface area correction: standardized eGFR)<br>6. Patients with a history of acute or chronic liver disease other than NAFLD/NASH and complications:<br>(1) Patients suffering from hepatitis B (defined by hepatitis B surface (HBs) antigen positive at the time of screening) or hepatitis C (defined by hepatitis C virus (HCV) antibody positive at the time of screening). However, anti-HCV antibody positive patients who are judged to be negative for hepatitis C virus ribonucleic acid (HCV-RNA) can be registered if they can be confirmed to be negative for at least one year before screening.<br>(2) Patients with autoimmune hepatitis.<br>(3) Patients with primary biliary cholangitis, primary sclerosing cholangitis, Wilson's disease, α1-antitrypsin deficiency, hemochromatosis or iron overload, drug-induced or alcoholic liver disease, or a history of known biliary atresia. |

| | |
|---|---|
| | (4) Patients with suspicion or definitive diagnosis of hepatocellular carcinoma.<br>7. Patients with a history of human immunodeficiency virus (HIV) infection.<br>8. Patients with findings of portal hypertension (complications: ascites, hepatic encephalopathy, varicose veins, splenomegaly).<br>9. Patients with a history of NAFLD-related drugs (amiodarone, methotrexate, systemic glucocorticoids, tetracycline, tamoxifen, higher doses of estrogen, anabolic steroids or valproic acid than used for hormone replacement) or other hepatotoxins for at least 4 weeks prior to screening.<br>10. Patients who have used the following drugs:<br>(1) Patients who used insulin, glucagon-like peptide-1 (GLP-1) receptor agonists, SGLT2 inhibitors, or thiazolidine 12 weeks before screening,<br>(2) Patients who used ursodeoxycholic acid or vitamin E 12 weeks before screening,<br>(3) Patients whose doses of dyslipidemia drugs or antihypertensive drugs were changed 12 weeks before screening,<br>(4) Patients whose dose of oral diabetes treatment drug (dipeptidyl peptidase 4 [DPP-4] inhibitor, sulfonylurea [SU] preparation, α-glucosidase inhibitor, metformin) was changed 12 weeks before screening,<br>(5) Patients who used drugs known to have a significant effect on body weight (including over-the-counter drugs for weight loss) 12 weeks before screening,<br>(6) Patients using central nervous system depressants (barbital, sodium thiopental, morphine hydrochloride hydrate, brotizolam, diazepam, etc.).<br>11. Patients with 10% weight change 24 weeks before screening.<br>12. Patients scheduled to undergo surgery after obesity surgery (such as gastroplasty and Roux-en-Y gastric bypass surgery) or during the trial period.<br>13. Patients with a history of type 1 diabetes.<br>14. Patients with hemoglobin A1c (HbA1c) > 9.5% at screening or with uncontrolled type 2 diabetes.<br>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.<br>16. Patients with a history of New York heart association functional classification (NYHA classification) class III or IV heart failure due to factors other than hypertension.<br>17. Patients with a history of myocardial infarction, unstable angina, percutaneous coronary intervention, coronary artery bypass grafting, or stroke or major surgery 24 weeks before screening.<br>18. Patients with a history of substance abuse. |

| | |
|---|---|
| | 19. Patients with malignant tumors. However, patients who have undergone radical surgery, patients who have completed chemotherapy/radiation therapy, and patients who are undergoing hormone therapy can be registered.<br>20. Patients with known intolerance to MRI or patients who are contraindicated for MRI examination.<br>21. Other patients who the principal investigator or sub-investigator deems inappropriate for conducting this clinical trial. |
| Active ingredient name and dose of investigational drug | A group: WY-8678 (guanabenz acetate) 4 mg/day<br>B group: WY-8678 (guanabenz acetate) 8 mg/day |
| Administration method, observation and administration period | The study consists of an 8-week screening period, a 16-week treatment period, and a 4-week follow-up (the final timing of administration of the investigational drug is the morning of the V7 (16-week) visit). The investigational drug is orally administered twice daily for 16 weeks. |
| Endpoints | **Efficacy**<br>Primary endpoint:<br>● Percentage of those where the liver fat content (%) measured by MRI-PDFF at 16 weeks decreased by ≥ 3.46% from baseline (%)<br><br>Secondary endpoints:<br>1) Amount of change and rate of change from baseline in the measured values of the following items at 16 weeks. Here, the rate of change is defined as (value at 16 weeks - baseline value) / (baseline value).<br>● Percentage of those where the liver fat content (%) measured by MRI-PDFF at 16 weeks decreased by 3.46% or more from baseline for 4 mg group and 8 mg group (%)<br>● Amount of change and rate of change in liver fat content measured by MRI-PDFF<br>● Rate of change in ALT, AST, gamma-glutamyl transferase (γ-GTP)<br>● Rate of change in weight<br>● Rate of change in blood lipids (chylomicron cholesterol, chylomicron triglyceride, lipoprotein cholesterol, low-density lipoprotein [LDL] triglyceride, very low-density lipoprotein [VLDL] cholesterol, VLDL triglyceride, free cholesterol, apoprotein A1, apoprotein B, adipsin, free fatty acid)<br>● Rate of change in insulin resistance (HOMA-IR)<br>● Rate of change in liver hardness (MRE)<br>● Rate of change in fibrosis markers (enhanced liver fibrosis [ELF] score, Fibrosis-4 [FIB-4])<br>2) Search for new markers related to liver disease and obesity metabolic disease.<br><br>**Safety**<br>● Occurrence rate of adverse events |

| | |
|---|---|
| Major statistical methods | **Analysis set**<br><br>1. Efficacy analysis set<br>The main analysis set for efficacy evaluation is the full analysis set (FAS).<br>2. Safety analysis set<br>The main analysis set for safety evaluation is the safety analysis set (SAS).<br><br>**Data handling**<br>As a general rule, missing and outliers are not supplemented or excluded, and are used as they are. However, if there are cases that require special consideration before the data is fixed, the handling will be decided by the case review committee. In addition, the final treatment of variables related to timing in the analysis is specified in the statistical analysis protocol.<br><br>**Statistical analysis protocol**<br>1. Primary endpoint<br>The main analysis is for the FAS. A point estimate was calculated for the proportion of subjects whose "liver fat content (%) measured by MRI-PDFF at 16 weeks decreased by 3.46% or more from baseline", and a 90% Clopper-Pearson confidence interval is created for it. The following hypothesis test is then performed:<br>If the lower limit of the 90% Clopper-Pearson confidence interval 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, $\theta$ is the probability that the amount of change in liver fat content from baseline $\leq -3.46\%$.<br><br>2. Secondary endpoints<br>For each secondary endpoint, each group is summarized using descriptive statistics and parallel-group comparison is performed using t-test.<br><br>**Significance level and confidence coefficient**<br>This clinical trial is exploratory and does not set any specific statistical hypothesis. When performing a test or interval estimation for exploratory purposes, the significance level is 5% (total of upper and lower) and the confidence coefficient is 95%. Multiplicity is not considered for the test and the interpretation of confidence intervals. |
| Compliance with Good Clinical Practice (GCP) standards | This clinical trial is conducted in compliance with the Declaration of Helsinki, clinical trial protocol, criteria specified in "Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices" Article 14-3 and Article 80-2, and "Guideline for Good Clinical Practice" (GCP). Documents and records related to all clinical trials should be properly stored in each responsible department. |

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Clinical trial schedule:

| | Consent acquisition | Screening | Treatment period | | | | | | Follow-up |
|---|---|---|---|---|---|---|---|---|---|
| | | V1 | V2 | V3 | V4 | V5 | V6 | V7／EOT | V8 |
| Week | | Within 8 weeks prior to registration | Prior to start of administration | 2 weeks | 4 weeks | 8 weeks | 12 weeks | 16 weeks | 4 weeks after end of administration |
| Tolerable range | | -8 weeks | - | ±3 days | ±7 days | ±7 days | ±7 days | ±7 days | ±7 days |
| Consent acquisition | ○ | | | | | | | | |
| Selection criteria | | ○ | ◯ | | | | | | |
| Subject background | | ○ | | | | | | | |
| Serological test [a] | | ○ | | | | | | | |
| Chest X-ray | | ○ | | | | | | | |
| electro-cardiogram | | ○ | | | | | | | |
| Physical examination [b] | | ○ | ◯ | | | | | ○ | |
| Vital signs c | | ○ | ◯ | ◯ | ○ | ○ | ○ | ○ | ○ |
| Subjective and objective symptoms | | | ◯ | ◯ | ◯ | ◯ | ◯ | ◯ | ◯ |
| Pregnancy test [d] | | | ◯ | | | | | ◯ | |
| MRI[e] | | ○ | | | | | | ○ | |
| Liver biopsy | | △ | | | | | | | |
| Randomization | | | ○ | | | | | | |
| Hematology test / urine test [f] | | ○ | ◯[i] | | | | | ○ | ○ |
| Endocrinological examination | | ○ | | | | | | | |
| Biochemical test 1 | | ○ | ○[i] | | ○ | ○ | ○ | ○ | ○ |
| Biochemical test 2[g] | | ○ | ○[i] | | | | | ○ | |
| Other [h] | | | ○ | | | | | ○ | |
| Somatic cell genetic test | | | ● | | | | | | |
| Providing drugs | | | ○ | | ○ | ○ | ○ | | |
| Checking the medication status | | | | ◯ | ○ | ○ | ○ | ○ | |
| Survey of combination drugs | | ○ | ○ | ◯ | ○ | ○ | ○ | ○ | |
| Investigation of adverse events | | | | ◯ | ○ | ○ | ○ | ○ | ○ |

○: Implemented
△: Information is collected for cases with liver biopsy results (within 32 weeks before screening).
●: Genetic testing is an essential test.
a: Contains HBs antigen, HCV antibody and HCV-RNA.
b: Includes height (V1 only) and weight. BMI (V1) is calculated based on height and weight.
c: Vital signs include blood pressure, pulse rate, and axillary body temperature.
d: For women of childbearing potential, a urine pregnancy test will be performed on V2 and V7.
e: Use magnetic resonance imaging (MRI) to measure MR elastography (MRE) and liver fat (PDFF). Patients terminating before V7 (16th week) should undergo MRI at the end of treatment if they have completed at least 4 weeks of treatment.
f-h: Refer to Table 9.1-2 Clinical laboratory items
i: If there is data within 4 weeks, they can be substituted.

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## List of definitions of abbreviations and terms

Abbreviations

| Abbreviations | Expressions or explanations not abbreviated | |
|---|---|---|
| | English term | Japanese name or explanation |
| CTCAE | Common terminology criteria for adverse events | Common terminology criteria for adverse events |
| DPP-4 | Dipeptidyl peptidase-4 | Dipeptidyl peptidase-4 |
| EDC | Electronic data capture | Electronic data capture |
| EOT | End of treatment | End of treatment |
| FAS | Full analysis set | Full analysis set |
| GCP | Good clinical practice | Good clinical practice |
| HIV | Human immunodeficiency virus | Human immunodeficiency virus |
| ICH | International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use | International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use |
| MedDRA ／ J | Medical dictionary for regulatory activities/J | Medical dictionary for regulatory activities/J |
| MRE | Magnetic resonance elastography | Magnetic resonance elastography |
| NAFL | Non-alcoholic fatty liver | Non-alcoholic fatty liver |
| NAFLD | Non-alcoholic fatty liver disease | Non-alcoholic fatty liver disease |
| NASH | Non-alcoholic steatohepatitis | Non-alcoholic steatohepatitis |
| NASH CRN | NASH clinical research network | NASH clinical research network |
| NYHA 分類 | New York heart association functional classification | New York heart association functional classification |
| POC | Proof of concept | Proof of concept |
| PPS | Per protocol set | Per protocol set |
| SAS | Safety analysis set | Safety analysis set |
| SGLT2 | Sodium-glucose cotransporter | Sodium-glucose cotransporter |

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Inspection-related item abbreviation

| Abbreviation | Expressions or explanations not abbreviated | |
|---|---|---|
| | English term | English term |
| Alb | Albumin | Albumin |
| ALP | Alkaline phosphatase | Alkaline phosphatase |
| ALT | Alanine aminotransferase | Alanine aminotransferase |
| AST | Aspartate aminotransferase | Aspartate aminotransferase |
| BMI | Body mass index | Body mass index |
| BUN | Blood urea nitrogen | Blood urea nitrogen |
| CRP | C-reactive protein | C-reactive protein |
| CK | Creatine kinase | Creatine kinase |
| CK-18 | Cytokeratin 18 | Cytokeratin 18 |
| Cl | Chlorine | Chlorine |
| eGFR | estimated glomerular filtration rate | estimated glomerular filtration rate |
| FFA | Free fatty acid | Free fatty acid |
| FIB-4 | Fibrosis-4 | Fibrosis-4 |
| FT3 | Triiodothyronine | Triiodothyronine |
| FT4 | Thyroxine | Thyroxine |
| γ-GTP | γ-glutamyl transpeptidase | γ-glutamyl transpeptidase |
| GLP-1 | Glucagon-like peptide-1 | Glucagon-like peptide-1 |
| Hb | Hemoglobin | Hemoglobin |
| HbA1c | Hemoglobin A1c | Hemoglobin A1c |
| HBs | Hepatitis B surface | Hepatitis B surface |
| HCV | Hepatitis C virus | Hepatitis C virus |
| HCV-RNA | Hepatitis C virus ribonucleic acid | Hepatitis C virus ribonucleic acid |
| HDL-C | High-density lipoprotein-cholesterol | High density lipoprotein-cholesterol |
| HOMA-IR | homeostasis model assessment of insulin resistance | homeostasis model assessment of insulin resistance |
| Ht | Hematocrit | Hematocrit |
| IL-6 | Interleukin-6 | Interleukin-6 |
| INR | International normalized ratio | International normalized ratio |
| K | Potassium | Potassium |
| LDH | Lactate dehydrogenase | Lactate dehydrogenase |
| LDL-C | Low-density lipoprotein-cholesterol | Low-density lipoprotein-cholesterol |
| LBP | LPS binding protein | LPS binding protein |
| M2BPGi | Mac2 Binding Protein Glucosylation Isomer | Mac2 Binding Protein Glucosylation Isomer |
| MRI | Magnetic resonance imaging | Magnetic resonance imaging |
| Na | Sodium | Sodium |
| NAS | NAFLD activity score | NAFLD activity score |
| PDFF | Proton density fat fraction | Proton density fat fraction |
| pH | Potential of hydrogen | Potential of hydrogen |
| PNPLA3 | Patatin-like phospholipase domain containing 3 | Patatin-like phospholipase domain containing 3 |
| PT | Prothrombin time | Prothrombin time |
| PT-INR | Prothrombin time-International normalized ratio | Prothrombin time-International normalized ratio |
| PIIIP | Procollagen III peptide | Procollagen III peptide |
| T-Bil | Total bilirubin | Total bilirubin |
| TG | Triglyceride | Triglyceride |
| TIMP-1 | Tissue inhibitor of metalloproteinases-1 | Tissue inhibitor of metalloproteinases-1 |
| TMAO | Trimethylamine N-oxide | Trimethylamine N-oxide |
| TM6SF2 | Transmembrane protein 6 superfamily member 2 | Transmembrane protein 6 superfamily member 2 |
| TNF-α | Tumor necrosis factor-α | Tumor necrosis factor-α |
| TP | Total protein | Total protein |
| VLDL | Very low-density lipoprotein | Very low-density lipoprotein |

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Iwaki M, *et al. BMJ Open* 2022; 12:e060335. doi: 10.1136/bmjopen-2021-060335

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Clinical trial implementation system

Appendix

Patient diary

# 1 Development history

## 1.1 Origin and history of development

Non-alcoholic fatty liver disease (NAFLD) is a condition in which fatty liver is found by histological diagnosis or diagnostic imaging. Other liver diseases, such as alcoholism, are excluded. NAFLD is regarded as a phenotype of metabolic syndrome in the liver and is often associated with obesity, diabetes, dyslipidemia, and hypertension. The prevalence of NAFLD in Japan has increased from 12.9% in 1994 to approximately 34.7% in 2000, and is on the rise worldwide, including in Japan. NAFLD is classified as non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) with inflammation and 10-20% of NASH cases progress and lead to liver cancer and cirrhosis[1)].

It has been reported that the cause of the pathogenesis and progression of NASH is the effect of lipotoxicity due to the accumulation of cholesterol and triglyceride flowing into the liver in the liver [1)]. In addition, the progression of NASH fibrosis is considered to be an important factor in patients with liver cancer. As a treatment method, diet and exercise therapy with a low-calorie diet are effective, and it has been reported that weight loss improves liver function and liver histology [2)]. As a drug treatment, thiazolidine-based drugs are useful in large-scale randomized controlled trials for patients with diabetes, and improvement of liver function and liver histology has been confirmed [3)]. Hydrogenoxymethylglutaryl-coenzyme A (HMG-CoA) coenzyme inhibitor [4)] and ezetimibe have been reported to be useful for patients with dyslipidemia, but the evidence is insufficient [5)]. If a patient has complications of hypertension, the administration of angiotensin II receptor blocker is recommended because it can be expected to suppress inflammation and fibrosis of liver tissue [6)]. In addition, the antioxidant effect of vitamin E is useful for the development of the pathological condition of NAFLD / NASH, and further verification is required [7)]. On the other hand, hepatoprotective agents, such as ursodeoxycholic acid and glycyrrhizin, did not show any obvious improvement in NAFLD / NASH and are not recommended [8)]. In Europe and the United States, a meta-analysis documented improvements of hepatic fattening and hepatic fibrosis by weight loss surgery [9)]. However, there has been no data from Japan, and further examination is required. There is no unified view on the efficacy of NAFLD / NASH for any of the drugs, and there are currently no drugs covered by insurance for NAFLD / NASH globally, including Japan.

## 1.2 Summary of clinically important findings

Guanabenz acetate is a substance that has a selective α2-adrenergic receptor stimulating effect. It is used 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.

In recent years, separate from the aforementioned effects on the nervous system, guanabenz acetate binds to Helicase With Zinc Finger 2 (Helz2; also known as peroxisome proliferator-activated receptor-gamma [PPARγ] DNA binding domain Interacting Protein 1; PDIP1), which is one of the transcriptional coupling factors that regulate gene activity for specific nuclear transcription factors. Helz2 is thought to act as a coactivator by binding to the DNA binding region of the PPARγ nuclear transcription factor, which acts as a master regulator of metabolic regulation. The binding of guanabenz to Helz2 reduces the activity of Helz2 in the liver is reduced. As a consequence, gene regulation is altered, the leptin receptor (LepRb) promoter is activated, and LepRb expression in the liver is increased. The downstream signal AMP-activated protein kinase is then activated, reducing insulin resistance caused by obesity. As a result, efficacy in various clinical obesity-related metabolic diseases is expected. However, in fact, in obese mouse model mice, oral administration of guanabenz increased LepRb expression in the liver, decreased body weight, and decreased insulin resistance as well as

associated hyperglycemia, fatty liver, and blood lipid (low-density lipoprotein-cholesterol [LDL-c] concentration) [10].

## 1.3 Results from non-clinical trials

The history of identifying guanabenz acetate is as follows. High-throughput screening of 1200 small molecule compounds that bind to and dissociate from Helz2 identified 14 compounds based on their affinity. When we examined these 14 compounds concerning their LepRb expression indices in cultured hepatocytes, we identified guanabenz acetate, which promoted LepRb expression in a concentration-dependent manner.

Regarding the pharmacological effects of oral administration of guanabenz acetate, the use of high-fat diet (HFD)-induced obese mice has been instructive. In a pharmacological study, the frequency of oral administration was considered based on the highest dose of clinically used guanabenz acetate (once daily) and the estimated efficacy of 12 hours (twice daily). The low, intermediate, and high doses of guanabenz acetate were set at 0.11, 0.32, and 0.96 mg/kg body weight (BW), respectively.

1）Findings on medical efficacy

Continuous oral administration of guanabenz acetate produced a dose-dependent decrease in metabolic parameters such as hepatic triglyceride (TG) content, hyperglycemia associated with insulin resistance, and blood levels of insulin and leptin in HFD-induced obese mice. However, the homeostasis model assessment beta (HOMAβ) level, which indicates endogenous insulin secretory capacity, was not significantly affected by any dose of guanabenz acetate. The aforementioned low dose of guanabenz acetate for 14 days also significantly reduced hepatic TG content and blood glucose levels. The test results are presented in Fig. 1.

clinical trial protocol 番号：YCU-21001

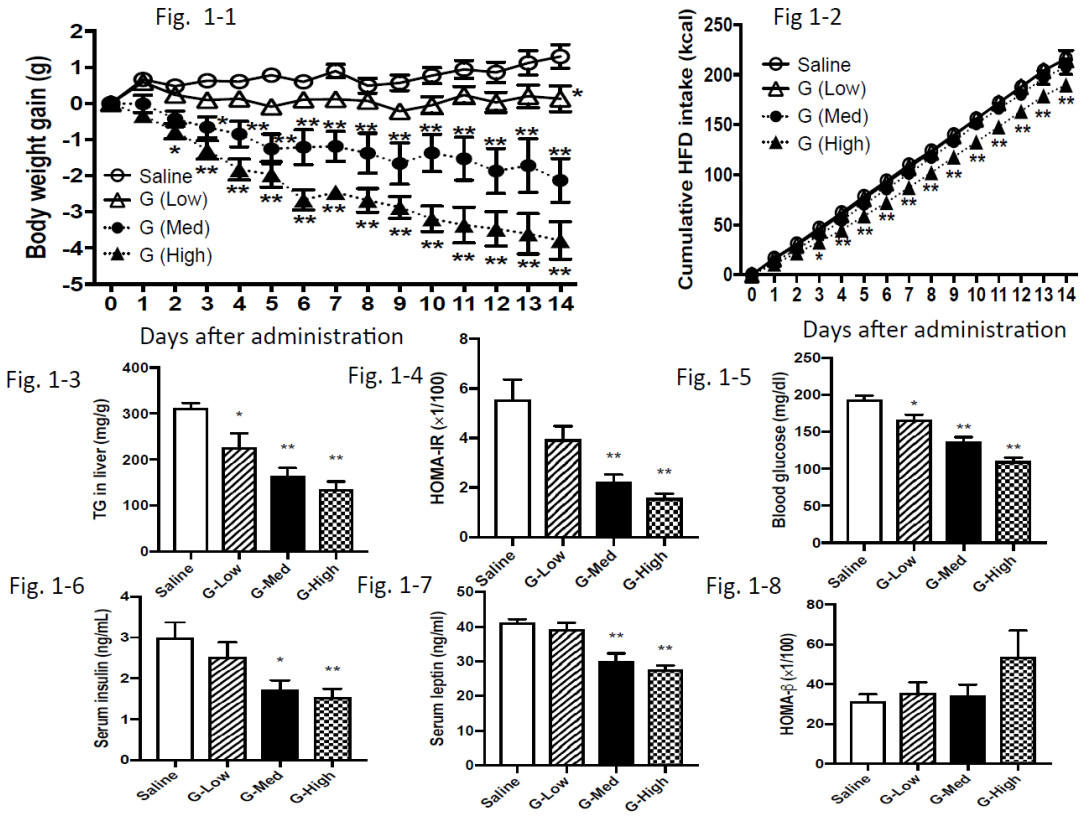



**Fig. 1.**

Weight gain from the start of administration (Fig. 1-1) and cumulative food intake (Fig. 1-2) (n = 7 in each group). Liver TG content after 14 days administration (Fig. 1-3), insulin resistance (Fig. 1-4), blood glucose level (Fig. 1-5), insulin (Fig. 1-6) leptin (Fig. 1-6, 1-7) and HOMA β value (Fig. 1-8). Saline, G-Low, G-Med, and G-High indicate saline treatment and low-dose, medium-dose and high-dose treatment of guanabenz acetate, respectively. The data are shown as mean ± standard error. * P <0.05 and ** P <0.01 compared with the saline treatment group.

2) Findings relating to safety

All HFD-induced obese mice treated with all doses of guanabenz acetate remained healthy. Although weight loss was observed, the mice did not develop diarrhea during the experimental period. A daily change in BW and food intake after administration of various doses of guanabenz acetate in HFD-induced obese mice revealed a dose-dependent weight loss at day 14 of the oral administration of guanabenz acetate (Fig. 1-1). On the other hand, a decrease in cumulative food intake was observed only after high-dose administration, and no significant change was observed in low-dose and medium-dose mice (Fig. 1-2). Helz2 is negligibly expressed in the brain, including the hypothalamus, which is the feeding center, and its complete absence does not affect feeding. Therefore, the decrease in food intake observed after administration of a high-dose, which is three times the medium dose, may not be related to Helz2 function, while the anorexia effect on the brain derived from the α2-adrenergic receptor stimulating action of this drug may cause decreased feeding and weight loss.

## 1.4 Experience of clinical use

Many drugs with different mechanisms have been used in the treatment of hypertension. Guanabenz acetate (WYTENS® tablets) acts by a central mechanism called α2 adrenergic receptor stimulating action involving the suppression of sympathetic nerve activity. However, this drug has been used for a long time since its launch in 1985 its use has been extremely limited. Use in patients with essential hypertension has been described, but there are no reports of experience in patients with non-alcoholic fatty liver disease, which is the subject of this clinical trial.

## 1.5 Summary of known and possible benefits and risks to the subject

### 1.5.1 Expected benefits of guanabenz acetate

In addition to improving fatty liver (NAFLD and NASH) associated with the improvement of insulin resistance induced by the action of guanabenz acetate on Helz2, improvement of diabetes, hyperlipidemia, and obesity is expected. In addition, improvement of hypertension based on the selective α2-adrenergic receptor stimulating action of guanabenz acetate is also expected.

### 1.5.2 Expected risks of guanabenz acetate

Guanabenz acetate suppresses peripheral sympathetic nerve activity by stimulating central sympathetic α2 receptors. Reported adverse drug reactions include dry mouth (2.9%) as well as neuropsychiatric symptoms (2.8%), such as drowsiness and dizziness.

At present, it is unclear whether there is an adverse drug reaction derived from the effect of this drug on Helz2 or the resulting improvement in insulin resistance. As long as this drug is used within the approved dosage range, it is unlikely that an as-yet unknown adverse drug reaction will occur. Therefore, the main risk in this study is considered to be the known adverse drug reactions associated with the suppression of sympathetic nerve activity. The subjects of this study are hypertensive patients with NAFL. Their background is different from the approved target patients (essential hypertension). Therefore, the frequency and severity of adverse drug reactions may differ.

When administering guanabenz acetate, adverse drug reactions based on the suppression of sympathetic nerve activity as described above may occur and must be kept in mind. In addition, it is difficult to specifically assume an adverse drug reaction derived from the action on Helz2 or the improvement of insulin resistance induced by it. However, it is necessary to carefully observe changes in metabolic parameters such as BW, blood glucose, and lipids, which are thought to have an effect, and to clarify the clinical profile of this drug.

# 2 Compliance with laws and regulations regarding GCP and this clinical trial

This clinical trial is conducted in compliance with the Declaration of Helsinki, clinical trial protocol, criteria specified in "Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices" Article 14-3 and Article 80-2, and "Guideline for Good Clinical Practice" (GCP). The implementation of the trial will comply with the ethical principles of the Declaration of Helsinki and maximize the human rights, welfare, and safety of the subjects.

# 3 Purpose of clinical trial

## 3.1 Purpose

We will investigate the efficacy and safety of 4 mg/day and 8 mg/day of WY-8678 (guanabenz acetate) in NAFLD/NASH patients with hypertension.

## 3.2 Phase of development

Phase IIa

# 4 Subjects

## 4.1 Subject disease

NAFLD/NASH

【Setting basis】

NAFLD in Japan affects 20 to 40% of adults. As a phenotype of metabolic syndrome in the liver, NAFLD is often associated with obesity, diabetes, dyslipidemia, hypertension, and other conditions. When it progresses to NASH, the risk of developing liver cirrhosis and liver cancer increases, and the risk of developing cardiovascular events is high. Therefore, preventing the progression of the condition by intervention is important. Rapid improvement of the pathological condition is required in NASH. If treatment with drugs becomes possible, it will lead to a significant decrease in patients with liver cirrhosis, liver cancer, and patients with cardiovascular events. 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 including NASH with hypertension should be set as a target group for which the medical efficacy for the pathological condition can be properly judged. This clinical trial is a clinical phase IIa study aimed at confirming the proof of-of-concept (POC) of guanabenz acetate therapy. We plan to examine the target and staging in the next phase.

## 4.2 Selection criteria

Subjects include patients who meet the following criteria at the time of screening.

1. Patients who have been fully informed about this study and have provided their written consent.
2. Patients aged ≥ 20 years and ≤ 75 years at the time of consent.
3. Patients diagnosed with essential hypertension and whose blood pressure at the time of screening is systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg (according to the diagnostic criteria for metabolic syndrome).
4. Patients diagnosed with NAFLD/NASH who meet the following criteria (1) or (2):
   (1) Patients diagnosed with NAFLD who meet the following three items
   ①Diagnostic imaging or histological detection of fatty liver,
   ②Alcohol intake < 30 g/day for men and < 20 g/day for women for at least 12 consecutive weeks in the 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
   * The definitive diagnostic criteria for NASH are defined as a fibrosis stage in liver biopsy in the evaluation using the "NASH Clinical Research Network (CRN) criteria" by an F1-F3 pathologist and a NAFLD activity score (NAS) ≥ 4 points (each item has one or more points):
   ① Fattening (0-3 points)
   ② Balloon-like swelling (0 to 2 points)
   ③ Inflammation in the lobules (0-3 points)
5. Patients with MRI-proton density fat fraction (PDFF) liver fat mass ≥ 8% at screening.
6. Patients with magnetic resonance elastography (MRE) value ≤ 3.6 kPa at screening.
7. Patients with BMI ≥ 25 kg/m$^2$ or more at the time of screening.
8. Patients receiving diet or exercise therapy 12 weeks before screening, with no improvement.
9. Patients who are willing to maintain a stable diet and physical activity during the clinical trial.

【Setting basis】

1. Ethical considerations

2. When voluntarily participating in a clinical trial, the person must be at least 20 years old for whom consent is legally established individually. In addition, considering the predominant age and safety of NAFLD/NASH, the upper limit is 75 years.
3. The disease is the target of the investigational drug. Blood pressure values set as the diagnostic criteria for metabolic syndrome associated with NAFLD are those formulated in 2005 by eight societies: Japan Atherosclerosis Society, Japan Diabetes Society, Japan Society for the Study of Obesity, Japan Society of Hypertension, Japanese Circulation Society, Japanese Society for Pediatric Nephrology, Japanese Society on Thrombosis and Hemostasis, and Japanese Society of Internal Medicine.
4. As a diagnostic criterion for NASH and considering the high risk of developing fibrosis, a NAS score ≥ 4, which has been widely defined in clinical trials internationally, was set. The diagnostic criteria for NAFLD were set in accordance with the 2014 NAFLD/NASH clinical practice guidelines.

5-7. Since the purpose of the clinical trial is to evaluate the effect of this investigational drug, it was set to set a certain standard for the target subjects in consideration of the effect on the efficacy evaluation.

8.9. The effects of changes in the dietary environment and exercise load on NAFLD pathology were minimized as much as possible.

## 4.3 Exclusion criteria

Patients who meet the following criteria at the time of screening are excluded:

1. Pregnant, lactating, potentially pregnant women, or patients who do not agree to contraception during the trial period.
2. 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 with drug allergies to guanabenz acetate.
4. Patients with liver failure or cirrhosis.
5. Patients with the following laboratory test values:
   (1) ALT > 430 IU/L (males) or > 240 IU/L (females); or AST > 300 IU/L
   (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 < 80,000/μL
   (5) eGFR < 45 (calculated by body surface area correction: standardized eGFR)
6. Patients with a history of acute or chronic liver disease other than NAFLD/NASH and complications:
   (1) Patients suffering from hepatitis B (HBs antigen positive at the time of screening) or hepatitis C (HCV antibody positive at the time of screening).
   HCV antibody-positive patients who are negative for HCV-RNA can be registered if they are confirmed to be negative for at least one year before screening.
   (2) Patients with autoimmune hepatitis.
   (3) Patients with primary biliary cholangitis, primary sclerosing cholangitis, Wilson's disease, α1-antitrypsin deficiency, hemochromatosis or iron overload, drug-induced or alcoholic liver disease, or a history of known biliary atresia.
   (4) Patients with suspicion or definitive diagnosis of hepatocellular carcinoma.
7. Patients with a history of HIV infection.
8. Patients with findings of portal hypertension (complications include ascites, hepatic encephalopathy, varicose veins, and splenomegaly).
9. Patients with a history of use of NAFLD-related drugs (amiodarone, methotrexate, systemic glucocorticoids, tetracycline, and tamoxifen, or higher doses of estrogen, anabolic steroids, or valproic acid than used for hormone replacement) or other hepatotoxins for at least 4 weeks prior to screening.
10. Patients who have used the following drugs:

(1) Insulin, glucagon-like peptide-1 (GLP-1) receptor agonists, sodium-glucose transport protein 2 (SGLT2) inhibitors, or thiazolidine 12 weeks before screening
(2) Ursodeoxycholic acid or vitamin E 12 weeks before screening
(3) Altered doses of dyslipidemia drugs or antihypertensive drugs 12 weeks before screening
(4) Altered dose of oral diabetes treatment drug (DPP-4 inhibitor, SU preparation, α-glucosidase inhibitor, metformin) 12 weeks before screening
(5) Those with 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% weight 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.
15. Patients with hyperthyroidism or hypothyroidism, or with screening results showing thyroid dysfunction
    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 New York Heart Association (NYHA) functional classification class III or IV heart failure due to factors other than hypertension.
17. Patients with a history of myocardial infarction, unstable angina, percutaneous coronary intervention, coronary artery bypass grafting, or stroke or major surgery 24 weeks before screening.
18. Patients with a history of substance abuse.
19. Patients with malignant tumors.
    Patients who have undergone radical surgery, patients who have completed chemotherapy/radiation therapy and patients who are undergoing hormone therapy can be registered.
20. Patients with known intolerance to MRI or patients who are contraindicated for MRI examination.
21. Other patients who the principal or sub-investigator deems inappropriate for conducting this clinical trial.

【Setting basis】

1. 3-8.16. 18-20 are set to ensure the safety of the subjects.
2.9.10(1)-(5).11-15 are set a certain standard for the target subjects after considering the influence on the safety assurance of the subjects and the evaluation of efficacy.
8.9. is set to eliminate the effects of other drugs in the accurate evaluation of efficacy.
10(6). is set to eliminate drugs that cause interaction by the medical efficacy of this investigational drug.
17. is set to ensure the safety of patients with complications that are expected to have an effect due to the medical efficacy and adverse drug reaction of this investigational drug.
21. In addition to the above items, this is set assuming that the principal investigator or sub-investigator is judged to be inappropriate for this clinical trial in light of scientific and ethical aspects.

## 5 Subject consent acquisition and information provision

### 5.1 Explanatory document / consent document

The principal investigator prepares explanatory and consent documents and amends them if necessary. The principal investigator obtains approval in advance from the institutional review board for the documents that are created and amended.

The following are kept in mind when creating an explanatory document:

1. Do not use words that cause the subject to waive their rights or consider doing so, or threaten/eliminate the legal liability of the principal investigator, sub-investigator, clinical trial collaborators, and involved medical institution.
2. Use non-technical terms as much as possible so that the subject can understand them.

### 5.2 Description

The principal investigator creates an explanatory document that includes at least the following contents:

1. That the clinical trial involves research
2. Purpose of the clinical trial
3. Principal investigator or sub-investigator name, job title, and contact information
4. Clinical trial method
5. Expected clinical benefits and risks or inconveniences
6. Presence or absence of other treatments and tests for the subject, and the expected significant benefits and risks associated with those treatments and tests.
7. Subject's planned participation period in the clinical trial
8. Statement that participation in the clinical trial is voluntary and that the subject may refuse treatment or withdraw from the clinical trial at any time. The refusal or withdrawal does not result in any adverse treatment of the subject or loss of benefits that would be incurred if the subject did not participate in the trial.
9. Monitors, auditors, institutional review boards, regulatory agencies, and other relevant individuals/groups should be able to view medical source documents with the subject's confidentiality preserved. The subject must provide a signed consent document to allow viewing.
10. Subject confidentiality should be preserved even when clinical trial results are published.
11. The contact person at the involved medical institution should respond if the subject wishes to obtain further information regarding the clinical trial and the subject's rights, or if there is a health hazard related to the clinical trial.
12. Compensation and treatment available to subjects in the event of clinical trial-related health hazards
13. Number of subjects planning to participate in the clinical trial
14. Statement that subjects will be promptly informed when information becomes available that may affect the subject's willingness regarding continued participation in the clinical trial
15. Conditions or reasons for terminating participation in clinical trials
16. If the subject needs to bear the cost, the details
17. If money is paid to the subject, the details
18. Responsibilities of the subject

### 5.3 Consent acquisition period and method

The principal investigator or sub-investigator should give the explanatory document and consent document approved by the institutional review board to the subject before the subject participated in this clinical trial, and fully explain the details. After answering questions from the subject and confirming that the subject fully understands the clinical trial, the subject voluntarily consents to

participate in the clinical trial by signing the document. The document also explains that the information obtained from participating in this clinical trial may be used for future studies. If this point is refused, the information will not be used for other research.

The principal investigator or sub-investigator who explained the details is also identified on the document and provides their seal on or signs the consent document. The date when the explanation was given to the subject is included in the document. If the clinical trial collaborators give a supplementary explanation, the clinical trial collaborators should also be registered, stamped, or signed, and the date should be entered.

### 5.4 Amendment of explanatory document/consent document

If any information is available that may affect the subject's will while the subject is participating in this trial, the principal investigator or sub-investigator must promptly convey the information to the subject, record the subject's intention to continue participation. If the principal investigator deems it necessary, the explanatory and consent documents are amended and approval is obtained from the institutional review board. In this case, the change will be explained using the board-approved amended document and consent document, and the subject’s signed re-consent will be obtained.

## 6 Subject registration

Participation in this clinical trial is by a registration system operated by the Case Registration Center. The registration procedure is detailed below.

### 6.1 Registration procedure

The principal investigator or sub-investigator obtains written consent from the candidate subject. The principal investigator or sub-investigator will enter the subject in the screening list and assign the subject identification code to the subjects who have provided their consent, and confirm their eligibility according to "**エラー! 参照元が見つかりません。 エラー! 参照元が見つかりません。**" and "**エラー! 参照元が見つかりません。 エラー! 参照元が見つかりません。**". If it is determined that there is no problem with eligibility, the principal investigator or sub-investigator, and clinical trial collaborators enter the necessary information into the electronic data capture (EDC) system and register at the time of V2. If there is no problem with eligibility, a case registration number will be issued. Registration is completed with this number. The details of the registration procedure are shown in the procedure manual specified separately.

### 6.2 Randomization method

At the time of enrollment, subjects will be assigned to one of the two groups described in "**エラー! 参照元が見つかりません。 エラー! 参照元が見つかりません。**" at a ratio of 1:1 using the allocation table prepared by the substitution block method. No adjustment factor is set. The applicable allocation number will be issued via the EDC system.

# 7 Investigational drug

## 7.1 Investigational drug code: WY-8678

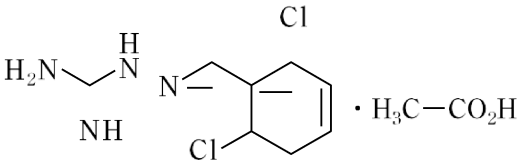
## 7.2 Active ingredient and content of investigational drug, dosage form, etc.

- Each tablet contains guanabenz acetate 2.525 mg (2 mg as guanabenz)
- White circular, scored, uncoated lock

## 7.3 Details of investigational drug

1.

Name: WYTENS® tablet, 2 mg
Generic name: guanabenz acetate
Chemical name: (E)-1-(2,6-Dichlorobenzylideneamino) guanidine monoacetate
Molecular formula: $C_8H_8Cl_2N_4 \cdot C_2H_4O_2$
Molecular weight: 291.13
Structural formula:




## 7.4 Packaging form and labeling

### 7.4.1 Packaging form

1. WY-8678

- Enclosed in one box (for each case) where WY-8678 is enclosed in a 5-pack in the following form:

4 mg/day
1 week: 14 tablets (1 tablet, 2 mg) per press-through package (PTP) sheet
4 weeks: 4 sheets per pack (aluminum packaging bag)
16 weeks + reserve drug (4 weeks): 5 packs per box per case

8 mg/day
1 week: 14 tablets (1 tablet, 2 mg), 2 PTP sheets
4 weeks: 8 sheets per pack (aluminum packaging bag)
16 weeks + reserve drug (4 weeks): 5 packs per box per case

### 7.4.2 Labeling

For 4 mg/day

**For clinical trial 4mg/day** Allocation number:

**WY-8678**

Content: 14 tablets × 4 sheets × 5 bags
Storage method: Room temperature
Serial number: GK27
Expiration date: 2025.9

Yokohama City University Hospital, Department of Palliative Medicine Clinical Instructor: Takaomi Kessoku

3-9 Fukuura, Kanazawa, Yokohama, Kanagawa 236-0004, Japan

For 8 mg/day

**For clinical trial 8 mg/day** Allocation number:

**WY-8678**

Content: 14 tablets × 8 sheets
Storage method: Room temperature
Serial number: GK27
Expiration date: 2025.9

Yokohama City University Hospital, Department of Palliative Medicine Clinical Instructor: Takaomi Kessoku

3-9 Fukuura, Kanazawa, Yokohama, Kanagawa 236-0004, Japan

Fig. 7.4.2-1 Labeling of WY-8678

## 7.5 Management of investigational drug

Shown separately in “Manual for investigational drug management”

# 8 Clinical trial design and clinical trial schedule

## 8.1 Clinical trial design

### 8.1.1 Clinical trial method

Single-center, randomized

### 8.1.2 Group composition

Subjects confirmed to be eligible during the screening period will be assigned to the following two groups at a ratio of 1 : 1 : 1 at the time of enrollment.

Group A: WY-8678 4 mg/day
Group B: WY-8678 8 mg/day

【Setting basis】

To determine drug efficacy two approved dose groups are set (4 mg/day and 8 mg/day). The efficacy of each group is evaluated instead of a parallel-group comparison.

### 8.1.3 Usage/dose of investigational drug, route of administration, administration period

During the treatment period, the investigational drug is orally administered to groups A and B twice daily for 16 weeks. The number of administered tablets in each group is as follows:

- Group: WY-8678, 4 mg/day: one tablet per session, twice a day
- B group: WY-8678, 8 mg/day: 2 tablets per session, twice a day

The investigational drug is first taken after dinner the day of visit 2 (V2). The final administration timing
should be the morning of V7 at 16 weeks.

【Setting basis】

- Dosage / administration method:

To explore the efficacy of guanabenz acetate, the approved doses of 4 mg and 8 mg were used. In a phase II pilot study, the antihypertensive effect of guanabenz acetate was slow and there was no significant difference in the incidence of adverse drug reactions between the 4 mg and 8 mg starting doses [11]. All of them are in the approved dose range. The doses were set because the clinical data showed that the start of administration from 8 mg also ensured sufficient safety. After confirming the POC in this study, we plan to investigate the appropriate dose of the drug in a Phase IIb study.

- Administration period:

Globally, short-term clinical trials for NAFLD (e.g., 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 Non-Alcoholic Fatty Liver Disease) 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 from image evaluation by MRI-PDFF is the primary endpoint. Therefore, it was considered that a certain administration period was required to confirm the efficacy of this drug, and 16 weeks was judged to be the appropriate period.

### 8.1.4 Scheduled participation period of subjects

The study consists of an 8-week screening period, a 16-week treatment period, and a 4-week follow-up (the final timing of administration of the investigational drug is the morning of V7 at 16 weeks). The investigational drug is orally administered twice daily for 16 weeks.

## 8.2 Clinical trial period (planned)

May 1, 2021 to June 30, 2023
(Consent acquisition period: September 1, 2021 to July 31, 2022)

## 8.3 Concomitant treatment

### 8.3.1 Drugs/treatments prohibited for concomitant use

1. The following drugs are prohibited from concomitant use from the time of consent acquisition to the treatment period:
   (1) Ursodeoxycholic acid
   (2) Guanabenz analogs (clonidine, methyldopa)
   (3) Thiazolidine, glucagon-like peptide-1 (GLP-1) receptor agonist, SGLT2 inhibitor, insulin
   (4) Central nervous system depressants (barbital, sodium thiopental, morphine hydrochloride hydrate, brotizolam, diazepam)
   (5) Vitamin E

(6) NAFLD-related drugs (amiodarone, methotrexate, systemic glucocorticoids, tetracycline, tamoxifen, higher doses of estrogen, anabolic steroids, or valproic acid than used for hormone replacement) or other hepatotoxins
(7) Drugs that significantly affect body weight (including over-the-counter weight loss drugs)

【Setting basis】
(1)(3)(5)-(7) were set because they may affect the evaluation of efficacy in this study.
(2) was set since it is the same active ingredient as the investigational drug, it affects the evaluation of efficacy in this study and ensures safety
(4) was set because there is a risk of enhancing or diminishing the action when used in combination with an investigational drug.

2. From the time of screening to the follow-up end, the following therapies are prohibited:
(1) Obesity surgery (sleeve gastrectomy, gastric bypass surgery, sleeve bypass surgery, etc.)

【Setting basis】
(1) was set because it is considered to affect the safety of the subjects and the efficacy evaluation of this study.

### 8.3.2 Continued use of pretreatment and combination therapy

When the following drugs and therapies are used in combination during the treatment period, they can be used under the conditions described.

1. Drugs restricted for concomitant use include:
(1) Antihypertensive drug
(2) Drugs to treat dyslipidemia
(3) Drugs to treat diabetes treatment (DPP-4 inhibitor, SU preparation, α-glucosidase inhibitor, metformin)

The above concomitant 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 is not changed until the end of the investment drug administration.
* Regarding antihypertensive drugs, if symptoms worsen, additional administration of only calcium antagonists is possible.
* Regarding diabetes treatment drugs, if the symptoms worsen, the dose can be gradually reduced.
The use of drugs for concomitant use after the end of the administration of the investigational drug is not restricted.

【Setting basis】
The above therapeutic agents are reportedly efficacious in patients with NAFLD/NASH. However, from the viewpoint of subject safety, it is difficult to designate these drugs as a concomitantly prohibited drug. Therefore, it was judged that there would be no problem in evaluating safety and efficacy if the subjects had stable pathological conditions with no change in dose from 12 weeks before screening and no change in dose during the study period.

2. Treatments restricted for concomitant use
Not applicable

## 8.4 Guidance to subjects

### 8.4.1 Medication guidance and compliance items

The principal investigator, sub-investigator, or clinical trial collaborators will give medication guidance to the subjects, paying attention to the following points at the time of delivery of this investigational drug:

1. When to take, how many times to take, and how to take; the investigational drug should be taken after dinner on the day of V2, and the final administration should be the morning of V7, at 16 weeks.
2. At the time of visit, the subject was expected to fast for 8 hours before the examination.
   ・If the clinic visit is in the morning, do not eat breakfast, take an investigational drug, and come to the hospital.
   ・If the clinic visit is later in the afternoon, eat breakfast, take the investigational drug, do not eat lunch, and come to the hospital.
   ・Breakfast should be skipped if necessary to comply with an 8-hour fast before the test.
3. If a dosing time was missed accidentally, bring any remaining medicine and an empty sheet of paper at the next visit.
4. If a dose is missed, take it at least 6 hours before the next dose.
5. If a subject is unsure about the medication, the subject should contact the principal investigator, sub-investigator, or clinical trial collaborators. The subject should be instructed to hand in their drug diary and fill in the medication status, daily status, etc.
6. While taking investigational drugs, subjects should be instructed to be careful when engaging in dangerous activities, such as work at heights and driving a car, and to minimize alcohol intake.

### 8.4.2 Recovery of drugs that are not taken

The principal investigator, sub-investigator, or clinical trial collaborators should recover any unconsumed drug from the subject. The drug should be given to the investigational drug administrator. If the unconsumed drug cannot be recovered, the reason should be provided in the medical record.

## 8.5 Overall flow of this clinical trial

### 8.5.1 Consent acquisition

The principal investigator or sub-investigator obtains written consent from the subject at the time of screening in accordance with “**エラー! 参照元が見つかりません。 エラー! 参照元が見つかりません。**” of this clinical trial protocol. After obtaining consent, the required items are completed in the "Subject Screening List".

### 8.5.2 Observation, inspection, and survey items during screening (V1)

The principal investigator or sub-investigator confirms that the subject complies with the provisions of "**エラー! 参照元が見つかりません。 エラー! 参照元が見つかりません。**" of this clinical trial protocol and does not violate the provisions of "**エラー! 参照元が見つかりません。 エラー! 参照元が見つかりません。**". If the subject is receiving medical treatment from another doctor, contact that doctor about the subject's participation in the clinical trial and record the contact in the medical record.

- Selection criteria
- Subject background
- Serological test
- Chest X-ray
- Electro-cardiogram
- Physical examination
- Vital signs

- MRI * (MRE, MRI-PDFF)
- Liver biopsy (information collection only for cases with data within 32 weeks before screening)
- Hematology/coagulation, urinalysis
- Endocrinological examination
- Biochemical test 1
- Biochemical test 2
- Combination drug

*: Measurements will be carried out by a specialist, such as a principal investigator. Eligibility will be judged by the principal investigator. Measurements by a specialist should be performed by the end of the trial.

#### 8.5.3 Pre-administration observation, examination, survey items (V2)

- Selection criteria
- Physical examination
- Vital signs
- Subjective and objective symptoms
- Pregnancy test
- Hematology / coagulation *, urinalysis *
- Biochemical test 1 *
- Biochemical test 2 *
- Others
- Genetic testing
- Concomitant drug

*If there is data within 4 weeks, it can be substituted.

#### 8.5.4 Two weeks after administration (V3)

- Vital signs
- Subjective and objective symptoms
- Medication status
- Concomitant drug
- Adverse events

#### 8.5.5 Observation, examination, survey items of 4 weeks (V4), 8 weeks (V5), and 12 weeks (V6) after administration

- Vital signs
- Subjective and objective symptoms
- Biochemical test 1
- Medication status
- Concomitant drug
- Adverse events

#### 8.5.6 Observation, examination, and survey items at 16 weeks of administration/termination) (V7/end of trial [EOT])

- Physical examination
- Vital signs
- Subjective and objective symptoms
- Pregnancy test
- MRI * (MRE, MRI-PDFF)

- Hematology/coagulation, urinalysis
- Biochemical test 1
- Biochemical test 2
- Others
- Medication status
- Concomitant drug
- Adverse events

* Measure by a specialist, such as a principal investigator. Measurements by a specialist should be performed by the end of the trial.

#### 8.5.7 Observation, examination, and survey items 4 weeks after the end of administration (follow-up, V8)

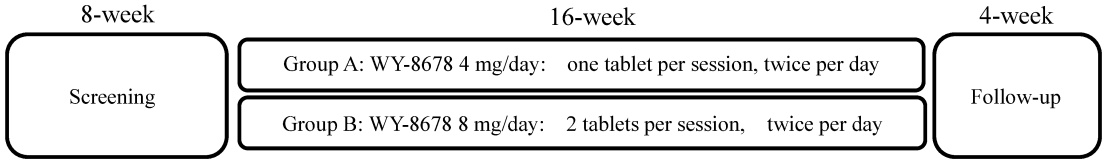
- Vital signs
- Subjective and objective symptoms
- Biochemical test 1
- Hematology/coagulation, urinalysis
- Adverse events

# 9 Observation, inspection, evaluation items, and timing

## 9.1 Observation, inspection, and evaluation schedule list

The principal investigator or sub-investigator conducts observations, inspections, surveys, etc. according to the table below. If a blood sampling test is to be performed at the time of the subject's visit, the subject should fast for 8 hours before the blood is collected.

Table 9.1-1 Observation, examination, and evaluation schedule




| | Consent acquisition | Screening | Treatment period | | | | | | Follow-up |
|---|---|---|---|---|---|---|---|---|---|
| | | V1 | V2 | V3 | V4 | V5 | V6 | V7／EOT | V8 |
| Week | | Within 8 weeks prior to registration | Prior to start of administration | 2 weeks | 4 weeks | 8 weeks | 12 weeks | 16 weeks | 4 weeks after end of administration |
| Tolerable range | | -8 weeks | - | ±3 days | ±7 days | ±7 days | ±7 days | ±7 days | ±7 days |
| Consent acquisition | ○ | | | | | | | | |
| Selection criteria | | ○ | ◯ | | | | | | |
| Subject background | | ○ | | | | | | | |
| Serological test [a] | | ○ | | | | | | | |
| Chest X-ray | | ○ | | | | | | | |
| Electro-cardiogram | | ○ | | | | | | | |
| Physical examination [b] | | ○ | ◯ | | | | | ○ | |
| Vital signs c | | ○ | ◯ | ◯ | ○ | ○ | ○ | ○ | ○ |
| Subjective and objective symptoms | | | ◯ | ◯ | ◯ | ◯ | ◯ | ◯ | ◯ |
| Pregnancy test [d] | | | ◯ | | | | | ◯ | |
| MRI[e] | | ○ | | | | | | ○ | |
| Liver biopsy | | △ | | | | | | | |
| Randomization | | | ○ | | | | | | |
| Hematology test / urine test [f] | | ○ | ◯[i] | | | | | ○ | ○ |
| Endocrinological examination | | ○ | | | | | | | |
| Biochemical test 1 | | ○ | ○[i] | | ○ | ○ | ○ | ○ | ○ |
| Biochemical test 2[g] | | ○ | ○[i] | | | | | ○ | |
| Other [h] | | | ○ | | | | | ○ | |
| Somatic cell genetic test | | | ● | | | | | | |
| Providing drugs | | | ○ | | ○ | ○ | ○ | | |
| Checking the medication status | | | | ◯ | ○ | ○ | ○ | ○ | |
| Survey of combination drugs | | ○ | ○ | ◯ | ○ | ○ | ○ | ○ | |
| Investigation of adverse events | | | | ◯ | ○ | ○ | ○ | ○ | ○ |

○: Implemented
△: Information is collected for cases with liver biopsy results (within 32 weeks before screening).
●: Genetic testing is an essential test.

a: Contains HBs antigen, HCV antibody, and HCV-RNA.

b: Includes height (V1 only) and weight. BMI (V1) is calculated based on height and weight.

c: Vital signs include blood pressure, pulse rate, and axillary body temperature.
d: For women of childbearing potential, a urine pregnancy test will be performed on V2 and V7.
e: Use MRI to measure MR elastography (MRE) and liver fat (PDFF). Patients terminating before V7 (week 16) should undergo MRI at the end of treatment if they have completed at least 4 weeks of treatment.

f-h: Refer to Table 9.1-2 Clinical laboratory items.
i: If there is data within 4 weeks, it can be substituted.

Table 9.1-2 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 (Alb)<br>ALT<br>Alkaline phosphatase (ALP)<br>Amylase<br>AST<br>Blood urea nitrogen (BUN)<br>Chlorine (Cl)<br>Creatinine (Cre)<br>Estimated glomerular filtration rate (eGFR) [3] (during screening)<br>γ-GTP<br>Lactate dehydrogenase (LDH)<br>Potassium (K)<br>Sodium (Na)<br>Calcium (Ca)<br>Total bilirubin (T-Bil)<br>Total protein (TP)<br>Uric acid | HDL-C<br>LDL-C<br>Non-HDL-C[3]<br>TC<br>TG<br><br>Glucose<br>HbA1c<br>Insulin<br>HOMA-IR[3] | [Inflammation]<br>High-sensitivity C-reactive protein (CRP)<br>Ferritin<br>TNF-a<br>Interleukin-6<br>CK18 / M30<br>Endotoxin LBP<br>Endotoxin activity<br>[Endocrine]<br>C-peptide<br>Total GLP-1 / Active GLP1<br>Leptin<br>Adiponectin<br>[Fibrosis]<br>hyaluronic acid<br>PIIIP<br>TIMP-1<br>M2BPGi<br>Type IV Collagen 7s<br>ELF score3<br>Fibrosis-4 (FIB-4) [3]<br>[Fat]<br>Chylomicron cholesterol<br>Chylomicron triglyceride<br>Lipoprotein cholesterol<br>LDL triglyceride<br>VLDL cholesterol<br>VLDL triglyceride<br>Free cholesterol (F-cho)<br>Apolipoprotein A1<br>Apolipoprotein B<br>Adipsin<br>Free fatty acid (FFA)<br>[Others]<br>TMAO |
| Hematological examination / coagulation (During screening, V2, V7, follow-up, termination) | Urinalysis (Screening, V2, V7, termination, follow-up) | |
| Hematocrit (Ht)<br>Hemoglobin (Hb)<br>Platelet count<br>Number of red blood cells<br>White blood cell count and white blood cell fraction (neutrophils, eosinophils, basophils, lymphocytes, monocytes)<br>International Normalized Ratio (INR) | Latent blood<br>Urine sugar<br>pH<br>Urine protein<br>Specific gravity<br>Urobilinogen<br><br>Pregnancy test [1]<br>(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<br>TM6SF2 | Free thyroxine (FT4)<br>Free triiodothyronine (FT3)<br>Thyroid stimulating hormone | HBs antigen<br>HCV antibody [2] |

1 Postmenopausal is defined as a condition without medical causes and no menstruation for more than 12 months.
2 Perform HCV-RNA test if HCV antibody is positive or if hepatitis C is present in the past.
3 According to the calculation formula.

## 9.2 Survey of subject background

The following background information of subjects participating in the clinical trial should be included:

- Subject identification code
- Sex
- Age at the time of consent
- Consent acquisition date
- Major underlying diseases/complications present in patient at the time of consent acquisition

- Drug allergy

## 9.3 Observation/examination items and endpoints related to efficacy evaluation

- Rate of change and amount of change in liver fat content (%) measured by MRI-PDFF at 16 weeks from baseline

- Rate of change from baseline in measurements at 16 weeks includes:
  1. ALT, AST, γ-GTP
  2. Weight
  3. Blood lipids (chylomicron cholesterol, chylomicron triglyceride, lipoprotein cholesterol, LDL triglyceride, VLDL cholesterol, VLDL triglyceride, free cholesterol, apoprotein A1, apoprotein B, adipsin, free fatty acid)
  4. Insulin resistance (HOMA-IR)
  5. Liver hardness (MRE)
  6. Fibrosis markers (ELF score, Fibrosis-4 (FIB-4))
- Rate of change of each clinical laboratory test value (inflammation, endocrine, fibrosis, etc.)

## 9.4 Observation/examination items and endpoints related to safety evaluation

- Occurrence rate of adverse events

## 9.5 Primary endpoint

- Percentage of those where the liver fat content (%) measured by MRI-PDFF at 16 weeks decreased by 3.46% or more from baseline (%)

【Setting basis】

To evaluate hepatic fattening of NAFLD, evaluation using MRI-PDFF is considered appropriate for the pathological condition.

Recent reports have indicated that the diagnostic ability of MRI-PDFF is superior to that of CAP as a diagnostic assessment of fattening of the liver 13). Liver biopsy is commonly used to assess hepatic fat mass. However, it is invasive and is not suitable for monitoring. Image evaluation by MRI-PDFF is stable and highly reproducible. In addition, since the judgment of fat deposition differs depending on the collection site in liver biopsy, MRI-PDFF, which enables easy quantitative evaluation, is considered appropriate 14) -17).

The 24-week MOZART placebo-controlled randomized controlled trial explored the correlation between histological changes by liver biopsy and hepatic fat content using MRI-DFF to examine the effect of ezetimibe on NASH. The trial reported an estimated cutoff point of -3.46% for changes in liver fat content from baseline that optimally distinguished between histological responders and non-responders 12). Patients with a change from baseline of ≤ -3.46% in hepatic fat content measured by MRI-PDFF were 4.3 times more likely of being a true histological responder than a false positive.

The purpose of this clinical trial is to confirm a POC. Based on this, we plan to conduct a study to confirm the dose setting in the next phase, and to conduct a verification test of efficacy by evaluation including liver biopsy (confirmation of fibrosis).

## 9.6 Secondary endpoints

1） Amount of change and rate of change from baseline in the measured values at 16 weeks were determined. The rate of change is defined as (value at 16 weeks - baseline value) / (baseline value). The following items were measured:

- Percentage of subjects where the liver fat content (%) measured by MRI-PDFF at 16 weeks decreased by ≥ 3.46% from baseline for the 4 mg and 8 mg groups
- Amount of change and rate of change in liver fat content measured by MRI-PDFF
- Rate of change in ALT, AST, and γ-GTP
- Rate of change in weight
- Rate of change in blood lipids (chylomicron cholesterol, chylomicron triglyceride, lipoprotein cholesterol, LDL triglyceride, VLDL cholesterol, VLDL triglyceride, free cholesterol, apoprotein A1, apoprotein B, adipsin, free fatty acid)
- Rate of change in insulin resistance (HOMA-IR)
- Rate of change in liver hardness (MRE)
- Rate of change of fibrosis markers (ELF score, Fibrosis-4 [FIB-4])

2） Search for new markers related to liver disease and obesity metabolic disease

## 10 Pharmacogenomics

### 10.1 Somatic genetic testing

This testing is conducted to evaluate the effects of disease susceptibility genes (PNPLA3, TM6SF2) in NAFLD on clinical endpoints and drug responsiveness. These assessments are exploratory and will not be included in the summary report.

#### 10.1.1 Blood sample collection time/sample processing

Blood samples are collected at the time specified in "**エラー! 参照元が見つかりません。 エラー! 参照元が見つかりません。**". Blood samples are processed by following the procedure manual specified separately. The total amount of blood collected is approximately 5 mL.

#### 10.1.2 Specimen storage and management

Specimens should be stored with the subject identification code. Specimens should be stored for up to 5 years after final analysis before disposal. The subject identification code is also assigned to the data generated from the sample.

Excess specimens will be stored until they are discarded under the control of an authorized person in the Yokohama City University School of Medicine Department of Gastroenterology and Hepatology. A comparison table of subject identification code and information that can directly identify the subject should be stored appropriately together with the sample management table.

#### 10.1.3 Use and disposal of specimens

Surplus samples are stored together with the sample management table for 5 years. Specimens that have passed the storage period should be discarded in anonymized form unless there is a specific reason to keep them.

If consent is withdrawn, the identification code of the subject is deleted and discarded appropriately. Specimens that have been stored for more than 5 years since the final analysis should be discarded unless there is a specific reason to keep them. Use of surplus samples for purposes other than the research purpose shall be approved by the relevant ethics review committee by creating a new research protocol.

#### 10.1.4 Withdrawal of consent for use of specimens

If the subject withdraws consent to use the provided sample, the sample will be disposed of and will not be used in the study. However, this does not apply if the research results have already been announced at the time of withdrawal of consent. If the measurement/analysis has already been performed, it will continue to be used to maintain the integrity of the existing analysis.

The principal investigator will confirm that the following points have been implemented:

- The withdrawal of the subject's consent regarding the use of the provided sample shall be reported immediately. If the sample collected from the subject is stored in the implementing medical institution, it should be immediately identified and disposed of. This process will be recorded in the trial information.
- The withdrawal of consent is immediately notified to the medical institution storing the specimen. The specimen is then disposed of, and a record of the disposal is made and submitted to the medical institution coordinating the study.
- The subject is informed that the specimen has been disposed of.
- The specimen must be discarded by the end of a specific period, as described in the explanatory document.

## 11 Ensuring subject safety

### 11.1 Basic items

When registering subjects, the selection and exclusion criteria must be met. Care is taken not to register subjects who are not included in this study.

During the clinical trial period, the principal investigator, sub-investigator, or clinical trial collaborators shall be in constant contact with the subject to ensure safety of the subject. In addition, when adverse events occur, the safety of the subject is ensured by providing appropriate medical care.

### 11.2 Definitions

#### 11.2.1 Definition of adverse event

Adverse events are any unwanted or unintended signs (including laboratory test values, abnormal vital signs), symptoms, or illness that occur during the trial. The causal relationship with the investigational drug does not matter.

Symptoms and diseases that have occurred before the start of the clinical trial should be recorded as complications in the case report form and should not be classified as adverse events. However, if complications worsen during the clinical trial period, they will be treated as an adverse event. The day when the deterioration is confirmed is the onset date of the adverse event. For events that have multiple symptoms (including signs and abnormal laboratory test values) and have a diagnosis/disease name, the diagnosis/disease name is recorded as an adverse event name in the case report form.

#### 11.2.2 Evaluation of adverse event

Principal investigators, etc. determine the severity of adverse events with reference to Table Table 11.2.2-1.

Table 11.2.2-1 Severity criteria

| Classification | Criteria |
|---|---|
| 1 Mild | Does not interfere with daily life (eating, sleeping, bathing, going out, work, exercise, etc.) |
| 2 Moderate | Interferes with daily life (eating, sleeping, bathing, going out, work, exercise, etc.) |

| | |
|---|---|
| 3 Advanced | Daily life (meal, sleep, bathing, going out, work, exercise, etc.) is impossible |

The "diagnosis name" is generally entered for the name of the adverse event, but if a definitive diagnosis cannot be made, use "sign" or "symptom". It is desirable to describe as much as possible corresponding to "ICH Medical dictionary for regulatory activities / J (MedDRA / J)".

### 11.2.3 Determination of causality

The principal investigator or sub-investigator determines the causal relationship with the investigational drug by referring to Table 11.2.3-1. Among adverse events, those for which a causal relationship with the investigated drug cannot be excluded are considered adverse drug reactions.

Table 11.2.3-1 Criteria for determining causality with investigational drug

| Classification | Criteria |
|---|---|
| 1. Can be denied | There is no reasonable possibility that the investigational drug caused the adverse event |
| 2. Cannot be denied | There is at least a reasonable possibility for the causal relationship between investigational drugs and adverse event |

### 11.2.4 Definition of serious adverse event

A serious adverse event is an adverse event that corresponds to the following:

- Death
- Possibility of death
- Hospital or clinic admission, or extension of hospital stay, required for treatment
- Injury
- Possibility of injury
- Serious issue according to the above items
- Congenital illness or abnormality in later generations

However, the following are excluded:

1）Hospitalization planned before participating in the clinical trial
2）Hospitalization not related to adverse events, such as examination purposes
3）Hospitalization within 24 hours for follow-up purposes only

### 11.2.5 Definition of important adverse event

For those who conduct their own clinical trials, regarding the adverse events (excluding serious adverse events) that occurred in this clinical trial, the adverse events that had to be terminated using investigational drugs are regarded as important adverse events.

## 11.3 Response when serious adverse event occurs

The principal investigator or sub-investigator will take appropriate measures when a serious adverse event occurs. The principal investigator will immediately report the details to the head of the medical institution coordinating the trial and the funder using the unified “(Medical) Form 12”. The detailed reporting procedure shall be described in the "Manual for handling safety information".

If a serious adverse event requires reporting to the Minister of Health, Labor and Welfare, it is reported within a specified date according to the "Manual for handling safety information".

## 11.4 Re-definition of adverse event into standard terms

The terms used by the principal investigator and others are coded to correspond to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)

Medical dictionary for regulatory activities/J (MedDRA/J) for all adverse events that occur. The MedDRA / J version shall be the latest available version or the previous version.

## 12 Termination criteria and procedure

### 12.1 Termination criteria

The principal investigator or sub-investigator terminates the participation of a subject enrolled in a clinical trial if any of the following apply:

1. When a subject requests to be withdrawn from the clinical trial.
2. If it is found after registration that the subject does not meet the inclusion criteria or conforms to one or more exclusion criteria
3. When drugs or therapies whose concomitant use is prohibited are administered.
4. When it is judged that it is difficult to continue the clinical trial due to the occurrence of adverse events or for other reasons.
5. When the continuation of the clinical trial is judged to be inappropriate by the principal investigator or others.

[Setting basis for termination criteria]

1. This is explained when obtaining consent and was set as the subject's right.
2. Subjects who should not be administered were set because the clinical trial should be terminated early.

3.4. Set for safety reasons.

5. Other than the above, termination can be based on the judgment of the principal investigator or sub-investigator.

### 12.2 Termination procedure

If the principal investigator or sub-investigator finds that a subject meets the termination criteria, this will be explained to the subject, the subject’s participation in the clinical trial will be terminated, and appropriate measures will be taken. If a subject is judged to be ineligible at the time of registration, it is not necessary to create a case report form, and enrollment in the clinical trial will be ended without going through termination. When a subject requests clinical trial termination, the subject's rights should be fully respected and appropriate efforts should be made to confirm the reason.

1. Termination procedure prior to taking the investigational drug

   If the clinical trial is terminated before the subject has taken the investigational drug, the principal investigator or sub-investigator should enter the termination date (the date on which the termination was determined) and the reason for termination in the case report form. In addition, unused investigational drugs will be collected at the time of the subject's visit.

2. Termination procedure after taking investigational drug

   Except when the cooperation of the subjects is not obtained, the administration status of the investigational drug (during the administration period) and the occurrence status of adverse events are investigated. Tests and evaluations specified at the time of termination are performed. The principal investigator and others fill in the termination date (the date when the termination was judged) and the reason for the termination in the case report form. If termination is performed during the administration period, the remaining drug and the opened empty box will be collected at the time of the subject's visit.

   The principal investigator or sub-investigator confirms the presence or absence of an adverse event. If an adverse event is found, the principal investigator or sub-investigator carries out a follow-up survey as a general rule.

# 13 Statistical analysis

## 13.1 Target number of cases

28 cases (WY-8678 4 mg group: 14 cases; 8 mg group: 14 cases)

【Setting basis】

As described in the setting basis of "9.5 Primary endpoint", the odds that the subject whose liver fat content changes from baseline ≤ -3.46% are truly histological responders are approximately 4.3 times the false positives. In this cutoff value setting, the true positive probability is 0.59 and the false positive probability is 0.25, and the following relationship holds. Therefore, when the probability of existence of a true histological responder is zero, the probability that the change in liver fat content from baseline ≤ -3.46% is 0.25.

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

Θ: Probability that 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. The possibility that the probability of existence of a true histological responder is greater than zero can be examined by non-invasive means by showing that the proportion of subjects with a change in liver fat content from baseline ≤ −3.46% is > 0.25. 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 $H_0: \theta \leq 0.25$ by a one-sided test at the 5% level. As a concrete alternative hypothesis for setting the power, we assume $H_0: \theta = \theta_1 = 0.5$, which corresponds to the existence probability of 0.75 for a true histological responder. The sample size required to obtain 80% power was calculated to be 28 cases.

In this study, dose groups of 4 mg and 8 mg were set. The main concern is the total active drug administration cases. Half of the required sample size was assigned to each dose group.

## 13.2 Analysis set

The analysis set is defined as follows and is determined before fixing the data for each case.

1. Full Analysis Set (FAS)
   Of all the randomized cases, the group excludes the cases that correspond to the items shown below.
   (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.
   (3) Cases in which no endpoints related to efficacy have been measured.
2. Per Protocol Set (PPS; analysis set conforming to clinical trial protocol)
   A population of FAS excluding cases with clinical trial protocol violations, such as ex post facto findings of inclusion criteria violations or use of drugs or treatments whose concomitant use is prohibited.
3. Safety Analysis Set (SAS)
   Group of cases in which the investigational drug was administered, even once.

### 13.2.1 Efficacy analysis set

The main analysis set for efficacy evaluation is the FAS.

#### 13.2.2 Safety analysis set

The main analysis set for safety evaluation is the safety analysis set (SAS).

### 13.3 Data handling

As a general rule, missing data and outliers are not supplemented or excluded, and are used as they are. However, if there are cases that require special consideration before the data is fixed, the handling will be decided by the case review committee.

### 13.4 Statistical analysis protocol

The outline of the protocol is detailed below. Details of the statistical analysis method are described in the separately created statistical analysis protocol. If there is a change from the original analysis plan, it is described in this plan, statistical analysis protocol, or general report.

#### 13.4.1 Analysis of primary endpoint

The main analysis is conducted on the FAS. A point estimate is calculated for the proportion of subjects whose "liver fat content (%) measured by MRI-PDFF at 16 weeks decreased by 3.46% or more from baseline", and a 90% Clopper-Pearson confidence interval is created for it. The following hypothesis test is then performed. If the lower limit of the 90% Clopper-Pearson confidence interval 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, $\theta$ is the probability that the amount of change in liver fat content from baseline $\leq -3.46\%$.

#### 13.4.2 Analysis of secondary endpoints

For each secondary endpoint, each group is summarized using descriptive statistics and parallel-group comparison is performed using t-test.

#### 13.4.3 Analysis of safety endpoints

In each group, the number and proportion of adverse events are calculated according to the event and the severity specified in Section 11.2.2.

#### 13.4.4 Subgroup review

A summary and parallel-group comparison of changes in liver fat content from baseline measured by MRI-PDFF, and changes in ALT, AST, γ-GTP, and MRE from baseline are conducted in the subgroup with and without NAFLD disease susceptibility genes (PNPLA3, TM6SF2) in NAFLD. The same analysis is performed for each group with and without the disease susceptibility gene.

When evaluating other subgroups, the details are described in the statistical analysis protocol.

#### 13.4.5 Interim analysis

Not applicable

### 13.5 Significance level and confidence coefficient

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.

### 13.6 Procedure for reporting deviations from statistical analysis protocol

If there is a deviation from the statistical analysis protocol, the details are entered into the analysis report.

## 14 Compliance with and deviation from clinical trial protocol, as well as changes and amendments

### 14.1 Compliance with clinical trial protocol

This clinical trial will be conducted in compliance with this clinical trial protocol.

### 14.2 Deviation from clinical trial protocol

The principal investigator or sub-investigator must not deviate from the clinical trial protocol (including deviations such as missing survey items) or make changes without obtaining written approval based on the preliminary review of the institutional review board. However, this does not apply if it is medically unavoidable, such as to avoid the subject's urgent danger.

If the principal investigator or sub-investigator deviates from the clinical trial protocol for any reason, all details are recorded.

To avoid the urgent danger of the subject if deviations occur, the principal investigator will create a document describing the reasons for not following the clinical trial protocol for other clinically unavoidable reasons. The deviation(s) and the reason(s) will be immediately reported to the institutional review board and other concerned agencies by the head of the implementing medical institution.

### 14.3 Amendment of clinical trial protocol and case report form

1. Those who conduct their own clinical trials amend clinical trial protocol and case report form samples as necessary when the following matters other than the administrative matters of the clinical trial (e.g., correction of wording, such as change of telephone numbers) apply:
   (1) To update important information for conducting a clinical trial properly, such as matters related to the quality, efficacy, and safety of investigational drugs
   (2) When changing the clinical trial protocol due to medically unavoidable circumstances
   (3) When the head of the implementing medical institution gives instructions for correction based on the opinion of the institutional review board
2. Obtain approvals can be granted from the institutional review board for amended or modified clinical trial protocols and case report form samples. However, this does not apply when changing the layout of the case report form sample.

# 15 Conclusion, termination, or suspension of clinical trial

## 15.1 Conclusion of clinical trial

1. After the clinical trial ends, the principal investigator reports to the head of the implementing medical institution that the clinical trial has ended and provides a written summary of the clinical trial results.
2. When the head of the implementing medical institution receives this report, the institutional review board is promptly notified in writing. As well, notification of the outline of the clinical trial results based on the report submitted by the principal investigator is provided to the institutional review board.

## 15.2 Overall termination or suspension of clinical trial

### 15.2.1 Termination or suspension of a clinical trial by the person conducting clinical trial

1. The criteria for terminating or suspending a clinical trial by a person conducting the clinical trial include:
   (1) Ethically or medically unavoidable circumstances occur, such as ensuring the safety of the subject
   (2) The clinical trial is deemed insignificant
   (3) When it is recognized that the principal investigator or implementing medical institution has hindered the proper clinical trial by violating the GCP Ministerial Ordinance, clinical trial protocol or various procedure manuals (except for other medically unavoidable cases to avoid the subject's urgent danger)
2. If the clinical trial is determined or suspended, the person conducting the clinical trial promptly reports to the director of the implementing medical institution and the regulatory agency in writing the details of the termination or suspension and the reason.

### 15.2.2 Termination or suspension of clinical trial at implementing medical institution

1. The criteria for terminating or suspending a clinical trial by implementing medical institution or principal investigator are as follows:
   (1) The principal investigator determines that the termination or suspension of the clinical trial is appropriate for some reason
   (2) Serious or continued non-compliance is found, according to the GCP Ministerial Ordinance, clinical trial protocol or various procedure manuals of implementing medical institution, principal investigator, sub-investigator or clinical trial collaborators
   (3) Approval of the clinical trial is revoked by the institutional review board of the implementing medical institution
   (4) The principal investigator becomes unable to conduct a clinical trial due to a transfer or other reason until a new principal investigator is selected and the necessary procedures are completed
2. When a clinical trial is terminated or suspended, the principal investigator promptly reports to the head of the implementing medical institution of the termination or suspension and the reason. The head of the implementing medical institution, in turn, reports in writing to the institutional review board that the termination or suspension was promptly performed and the reason for the termination.
3. The head of the implementing medical institution cancels matters that have already been approved by the institutional review board (including the termination or suspension of the clinical trial) in the continuation review of the clinical trial being conducted by the institutional review board. If notified of this, the principal investigator is promptly notified in writing of the instructions and resulting decisions of the head of the implementing medical institution, along with a copy of the dated document regarding the cancellation of the institutional review board and the reason.

# 16 Case report form

## 16.1 Individuals who create case report form

The principal investigator, sub-investigator, and clinical trial collaborators create case report forms for registered cases.

## 16.2 Case report form format

EDC is used as the case report form in this clinical trial. EDC requirements follow "Computerized system aptitude management guidelines for pharmaceutical and quasi-drug manufacturers and distributors", "Guidelines for the use of electromagnetic records and electronic signatures related to applications for approval or permission of pharmaceutical products, etc." (ER / ES guidelines), and other regulatory requirements.

## 16.3 Precautions when creating, changing, or revising case report form

1. Case report form content confirmation

(1) Creator of case report form

Those nominated by the person conducting the clinical trial will be trained by the principal investigator or sub-investigator and clinical trial collaborators on how to use EDC and how to input information. The case report form should be created only by those who have received this training and have been issued an account.

(2) Creation of case report form

The principal investigator or sub-investigator, and clinical trial collaborators create a case report form according to the input guide provided by the person conducting the clinical trial within the scope of authority set in the EDC. In addition, as a general rule, the input shall be made promptly after obtaining the subject information.

(3) Revision of case report form

If the principal investigator or sub-investigator, and clinical trial collaborators determine that the content of the case report form needs to be modified, modify it on the EDC, and enter the reason. The content of the correction, the person who made the correction, and the date of the correction are automatically recorded as electronic information.

(4) Electronic signature of the case report form

The principal investigator checks the contents of the case report form, confirms that there are no problems, and then digitally signs it on the EDC system.

2. Storage of case report form

The principal investigator stores an electromagnetic recording medium (CD-ROM, etc.) related to the contents of the case report form (including the audit trail).

## 17 Direct browsing of source documents, etc.

### 17.1 Scope of source documents

Concerning the source documents related to this clinical trial, as a general rule, the materials including the source data of this clinical trial are the source documents. If source data appears in more than one source document, the source documents that will adopt the data are identified by the start of the trial. They include:

1. Record of subject consent
2. Medical records (medical records, nursing records, etc.)
3. Test records (clinical test results, etc.)
4. Electronic media, such as MRI examinations and X-ray examinations
5. Investigational drug management table
6. Records filled directly by the subject in the patient diary
7. Documents or records related to clinical trials, including communication records
8. Others

### 17.2 Implementation of direct browsing

During investigations by monitors or auditors, institutional review boards, and regulatory agencies designated by those who conduct clinical trials, the principal investigator and head of the implementing medical institution that conducted this clinical trial accept this according to the procedure of implementing medical institution and provide medical records, etc. (source documents) related to this clinical trial for direct viewing.

## 18 Clinical trial quality control and quality assurance

### 18.1 Clinical trial quality assurance

1. 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.
2. The principal investigator and the head of the implementing medical institution will provide the necessary information, including viewing all clinical trial-related records, such as source documents, upon request from the auditor, institutional review board.

### 18.2 Clinical trial quality control

1. The principal investigator or sub-investigator creates a case report form for each case. If there is a deviation from the clinical trial protocol, the principal investigator or sub-investigator will record all those facts. For deviations that do not follow the clinical trial protocol for other medically unavoidable reasons to avoid the subject's urgent danger, the principal investigator will prepare a document stating the particular reason and immediately submit it to the head of the implementing medical institution.
2. The data in the case report form that is based on source documents must be consistent with the source documents. If there is any inconsistency with the source documents, the principal investigator creates a record explaining the content and reason.
3. The principal investigator guarantees that data such as case report forms are accurate and complete.
4. Monitoring will be based on the procedure manual specified separately to verify human rights, the fact that the safety and welfare of the subjects are protected, the fact that the clinical trial is conducted in compliance with the latest clinical trial protocol and GCP, and data that include case report forms reported by principal investigators and clinical trial-related records, such as source documents. As well, the accuracy and completeness of the forms will be confirmed by the monitor.

5. The principal investigator and the head of the implementing medical institution provide the monitor with the information necessary for monitoring, including viewing source documents such as medical records.

### 18.3 Data quality assurance

1. The auditor confirms that the quality control of the data has been performed according to the GCP, standard work procedure manual, clinical trial protocol, and other predetermined plans.
2. The person in charge of audit confirms the report from the auditor and approves it.

### 18.4 Data quality control

1. Data quality control is performed by the monitoring and data management departments in accordance with standard operating procedures.
2. The monitor confirms that a clinical trial that complies with the GCP, clinical trial protocol, and "Manual for investigational drug management" is being conducted. The creation of the case report form matches the source documents. The recorded contents of the case report form are consistent.
3. The department in charge of data management confirms inconsistencies and logical inconsistencies in the recorded contents of the case report form.
4. If there is a discrepancy between the recorded contents of the case report form and the source documents, or if there is an inconsistency or logical contradiction in the recorded contents of the case report form, the principal investigator examines the validity of the item and corrects the case report form as necessary.

## 19 Ethical considerations

### 19.1 Ethical conduct of clinical trial

This clinical trial must be conducted in compliance with ethical principles based on the Declaration of Helsinki and in compliance with Act on Pharmaceuticals and Medical Devices, GCP, and clinical trial protocol.

### 19.2 Institutional review board review

Prior to conducting this clinical trial, the institutional review board for the implementing medical institution or an institutional review board that allows investigation and deliberation in place of setting up an institutional review board in the implementing medical institution will review the feasibility of conducting the clinical trial from the viewpoint of ethical, scientific, and medical validity based on materials, such as the investigational drug summary, clinical trial protocol, and explanatory document/consent document.

### 19.3 Items concerning the protection of human rights of subjects

1. Principal investigators and others should pay sufficient attention to respect for the subject's voluntary actions and health management in accordance with the spirit of the Declaration of Helsinki when conducting this clinical trial.
2. Monitors, audit managers, and persons in charge who have access to personal information of the subjects by directly browsing source documents and other sources are responsible for protecting the subject's personal information. In addition, the person involved in this clinical trial will maintain the confidentiality of the subjects when publishing the content of the clinical trial in a medical journal or submitting materials to regulatory agencies.

## 20 Record keeping

### 20.1 Storage period

### 20.1.1 Individuals conducting clinical trial themselves

A person who conducts a clinical trial independently must retain the documents or records related to the clinical trial to be preserved until the latter date specified in item 1 or 2.

1. Date of approval for manufacturing and marketing of the investigational drug (if development is terminated, 3 years must elapse from the date when development termination was determined).
2. Date when 3 years have passed since the termination or end of the clinical trial

This point concerns the documents or records related to the clinical trial that the head of the implementing medical institution, the convener of the institutional review board, or the principal investigator should keep. When a document is no longer necessary to retain, the head of the implementing medical institution or the convener of the institutional review board (via the head of the implementing medical institution) will be notified. In addition, if the applicable individual is no longer affiliated with their originally affiliated medical institution, then appropriate measures shall be taken regarding the preservation of the record.

### 20.1.2 Implementing medical institution

The person responsible for storage designated by the head of the implementing medical institution shall retain the documents or records relating to the clinical trial to be retained in the implementing medical institution until the latter date specified in item 1 or 2.

However, if the person conducting the clinical trial decides that storage for a longer period of time is necessary, the storage period and storage method shall be discussed with the principal investigator. When storing records, each person in charge of storage shall be determined and stored.

1. Date of approval for manufacturing and marketing of the clinical trial product: if notification is received stating that materials related to development termination or clinical trial results will not be attached to the application form, then a date of 3 years since the notification was received is set.
2. A date of 3 years since the termination or end of the clinical trial is set.

### 20.1.3 Institutional review board

The convener of the clinical trial review committee must keep records of standard work procedures, list of members (including qualifications of each member), list of professions and affiliations of members, submitted documents, minutes of meetings and letters, and other information, until the latter date specified in item 1 or 2.

However, if the person conducting the clinical trial determines that storage for a longer period of time is necessary, the storage period and storage method shall be discussed with the person conducting the clinical trial. These records should be available at the request of regulatory agencies.

1. Date of approval for manufacturing and marketing of the clinical trial product: if notification is received stating that materials related to development termination or clinical trial results will not be attached to the application form, then a date 3 years since the notification was received is set.

Note: Since the person in charge of record-keeping of the implementing medical institution is supposed to keep it until 3 years have passed from the date of receiving the notification, the installer of the institutional review board should handle it in the same way.

2. A date of 3 years since the termination or end of the clinical trial is set.

### 20.2 Confirmation of stored materials

After the end of the clinical trial, the monitor confirms that the materials that need to be saved are saved by the principal investigator and the implementing medical institution, and specifies the document name and storage location. In that case, the principal investigator and the person in charge of document storage of the implementing medical institution will cooperate.

## 21 Payment of money

When paying the burden reduction cost to the subject, the provisions of the implementing medical institution are followed.

## 22 Health damage compensation and insurance

### 22.1 Health damage compensation

If any health hazard occurs to the subject as a result of this clinical trial, the principal investigator and others will provide treatment and take other necessary measures. Those who conduct clinical trials themselves shall establish a procedure manual and take measures, such as insurance, to compensate for the health damage caused to the subjects in connection with the clinical trial. Those who conduct clinical trials themselves will respond to the health hazards of the subjects according to the procedure manual.

### 22.2 Insurance

Those who conduct their own clinical trials take out the insurance necessary to prepare for health damage compensation. The implementing medical institution will take insurance and other necessary measures in case of health damage caused by medical malpractice.

## 23 Publication arrangements

1. Persons involved in the medical institution related to this clinical trial cannot disclose information provided by the trial administrator, information obtained as a result of the clinical trial to a third party without prior written consent.
2. When the information obtained from this clinical trial is to be disclosed to outside parties such as academic societies by the persons involved in the medical institution, the consent of the person conducting the clinical trial must be obtained in advance in writing.

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## 25 Clinical trial implementation system

For the clinical trial implementation system, refer to "enclosed sheet Clinical trial implementation system". The amendment of the enclosed sheet will be carried out separately from this clinical trial protocol.

(1) Implementing medical institution

Yokohama City University Hospital, Fukuura, Kanazawa, Yokohama, Kanagawa 236-0004, Japan

(2) Principal investigator

Takaomi Kessoku, MD, PhD, Department of Palliative Medicine, Yokohama City University Hospital, Yokohama, Japan

(3) Investigational drug provider/manufacturer

Toyo Seiyaku Kasei Co., Ltd.

(4) Clinical trial coordination office

Center for Novel and Exploratory Clinical Trial (Y-NEXT), Research and Development Support Office, Yokohama City University Hospital

(5) Monitor (Representative)

Clinical Development Department, SRD Inc.

Main business activities:

- Confirming requirements for investigators and medical institutions
- Monitoring clinical trials
- Checking case report forms against source documents, etc.
- Confirming investigational drug management at the site
- Confirming operations related to the completion of the clinical trial at the site
- Confirming the documents kept by the site and investigator
- Confirming the measures to be taken when an adverse event occurs
- Implementing other monitoring activities in accordance with GCP and standard operating procedures

(6) Data Management Manager

Data Science Department, SRD Inc.

Main business activities:

- Preparing sample case report forms (drafts)
- Constructing and operating the EDC system
- Operating the data management system and preparing reports on these operations

(7) Person in charge of statistical analysis

Data Science Department, SRD Inc.

Main business activities:

- Performing statistical analysis
- Preparing statistical analysis reports

(8) Responsible for auditing

Reliability Assurance Office, SRD Inc.

Main business activities:

- Preparing audit plans
- Auditing clinical trial systems and individual clinical trials
- Preparing audit certificates

(9) Investigational drug allocation manager

Center for Novel and Exploratory Clinical Trial (Y-NEXT), Yokohama City University Hospital, Yokohama, Japan

Main business activities:

- Confirming that new investigational drugs are sealed upon arrival at investigational sites and
- Confirming the delivery of new investigational drugs to the investigational sites
- Confirming consistency between the results of formulation studies and the investigational drug allocation chart

(10) Case Registration Center

Data Science Department, SRD, Inc.

Main business activities:

- Confirming eligibility of participants and reconfirming enrollment
- Notifying the investigator of full enrollment acceptability
- Informing the investigator of the allocation number if enrollment is possible

(11) Laboratory

Skylight Biotech Inc.

Main business activities:

- Measuring samples (lipid items)
- Reporting measurement results

SRL Corp.

Main business activities:

- Measuring samples (inflammation, fibrosis, lipid items)
- Reporting measurement results