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

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Version history

Version No.	Creation date
Ver 1.0	July 16, 2021
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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 (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** 1. Patients who have received a full explanation about this study and who have provided written consent. 2. Patients ≥ 20 years of age ≤ 75 years of age at the time consent was provided. 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) 4. Patients diagnosed with NAFLD/NASH who meet the following criteria (1) or (2) (1) Patients diagnosed with NAFLD who meet the following three criteria: ① Diagnostic imaging or histological evidence of fatty liver, ② Alcohol intake < 30 g/day for men and < 20 g/day for women for 12 or more consecutive weeks one 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 (0-2 points)

	③ Inflammation in the lobules (0-3 points) 5. Patients with magnetic resonance imaging (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 a body mass index (BMI) ≥ 25 kg/m^2 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. **Exclusion criteria** 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) Alanine aminotransferase (ALT) > 430 IU/L (males) or > 240 IU/L (female); or aspartate aminotransferase (AST) > 300 IU/L (males and females) (2) Prothrombin time-international normalized ratio (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) Estimated glomerular filtration ratio (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 (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. (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 human immunodeficiency virus (HIV) infection. 8. Patients with findings of portal hypertension (complications: ascites, hepatic encephalopathy, varicose veins, splenomegaly). 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. 10. Patients who have used the following drugs: (1) Patients who used insulin, glucagon-like peptide-1 (GLP-1) receptor agonists, SGLT2 inhibitors, or thiazolidine 12 weeks before screening, (2) Patients who used ursodeoxycholic acid or vitamin E 12 weeks before screening, (3) Patients whose doses of dyslipidemia drugs or antihypertensive drugs were changed 12 weeks before screening, (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, (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, (6) Patients using 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 hemoglobin A1c (HbA1c) > 9.5% at screening or with uncontrolled type 2 diabetes. 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 New York heart association functional classification (NYHA 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. However, 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 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 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** Primary endpoint: ● Percentage of those where the liver fat content (%) measured by MRI-PDFF at 16 weeks decreased by ≥ 3.46% from baseline (%) Secondary endpoints: 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). ● 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 (%) ● Amount of change and rate of change in liver fat content measured by MRI-PDFF ● Rate of change in ALT, AST, gamma-glutamyl transferase (γ-GTP) ● Rate of change in weight ● 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) ● Rate of change in insulin resistance (HOMA-IR) ● Rate of change in liver hardness (MRE) ● Rate of change in fibrosis markers (enhanced liver fibrosis [ELF] score, Fibrosis-4 [FIB-4]) 2) Search for new markers related to liver disease and obesity metabolic disease. **Safety** ● Occurrence rate of adverse events

Major statistical methods	**Analysis set** 1. Efficacy analysis set The main analysis set for efficacy evaluation is the full analysis set (FAS). 2. Safety analysis set The main analysis set for safety evaluation is the safety analysis set (SAS). **Data handling** 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. **Statistical analysis protocol** 1. Primary endpoint 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: 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, θ is the probability that the amount of change in liver fat content from baseline $\leq -3.46\%$. 2. Secondary endpoints For each secondary endpoint, each group is summarized using descriptive statistics and parallel-group comparison is performed using t-test. **Significance level and confidence coefficient** 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.

clinical trial protocol 番号：YCU-21001

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.

clinical trial protocol 番号：YCU-21001

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

clinical trial protocol 番号：YCU-21001

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

clinical trial protocol 番号：YCU-21001

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

Table of contents

clinical trial protocol 番号：YCU-21001

Enclosed sheet

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

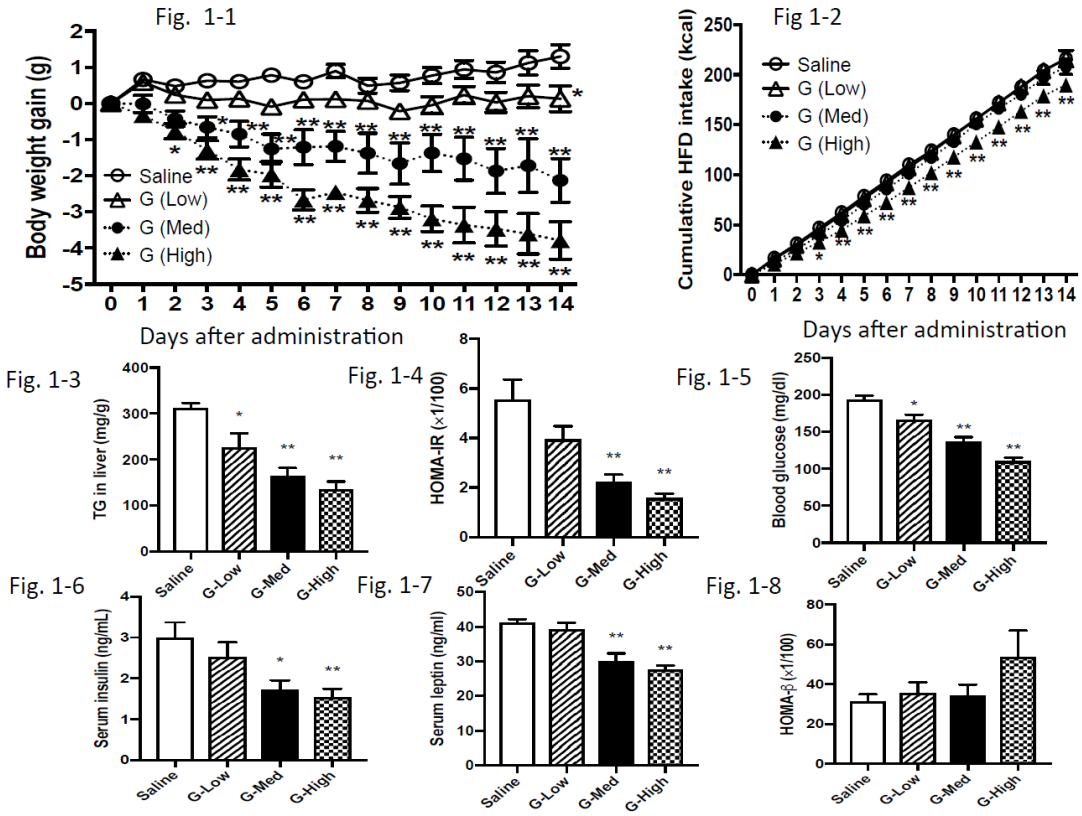


Fig. 1.

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

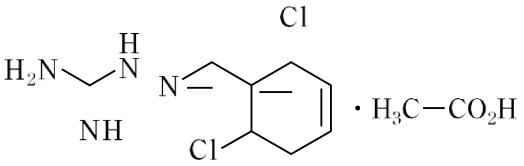
7.2 Active ingredient and content of investigational drug, dosage form, etc.

- 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)

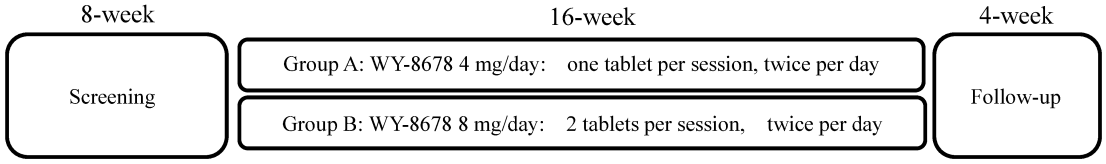
- Vital signs
- Subjective and objective symptoms
- Biochemical test 1
- Hematology/coagulation, urinalysis
- Adverse events

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) ALT Alkaline phosphatase (ALP) Amylase AST Blood urea nitrogen (BUN) Chlorine (Cl) Creatinine (Cre) Estimated glomerular filtration rate (eGFR) [3] (during screening) γ-GTP Lactate dehydrogenase (LDH) Potassium (K) Sodium (Na) Calcium (Ca) Total bilirubin (T-Bil) Total protein (TP) Uric acid	HDL-C LDL-C Non-HDL-C[3] TC TG Glucose HbA1c Insulin HOMA-IR[3]	[Inflammation] High-sensitivity C-reactive protein (CRP) Ferritin TNF-a Interleukin-6 CK18 / M30 Endotoxin LBP Endotoxin activity [Endocrine] C-peptide Total GLP-1 / Active GLP1 Leptin Adiponectin [Fibrosis] hyaluronic acid PIIIP TIMP-1 M2BPGi Type IV Collagen 7s ELF score3 Fibrosis-4 (FIB-4) [3] [Fat] Chylomicron cholesterol Chylomicron triglyceride Lipoprotein cholesterol LDL triglyceride VLDL cholesterol VLDL triglyceride Free cholesterol (F-cho) Apolipoprotein A1 Apolipoprotein B Adipsin Free fatty acid (FFA) [Others] TMAO
Hematological examination / coagulation (During screening, V2, V7, follow-up, termination)	Urinalysis (Screening, V2, V7, termination, follow-up)	
Hematocrit (Ht) Hemoglobin (Hb) Platelet count Number of red blood cells White blood cell count and white blood cell fraction (neutrophils, eosinophils, basophils, lymphocytes, monocytes) International Normalized Ratio (INR)	Latent blood Urine sugar pH Urine protein Specific gravity Urobilinogen Pregnancy test [1] (At V2, V7, at termination)	
Somatic cell genetic test (V2)	Endocrinological examination (at the time of screening)	Serological test (at the time of screening)
PNPLA3 TM6SF2	Free thyroxine (FT4) Free triiodothyronine (FT3) Thyroid stimulating hormone	HBs antigen HCV antibody [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, θ 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