Main Title

A 61-day Randomized, Double blind, Placebo-controlled Trial to Assess the Safety and Efficacy of Three Doses of HU6 in Subjects with Elevated Liver Fat and High Body Mass Index (28 to 45 kg/m^2)

Protocol Number:

RIV-HU6-203

Official Short Title:

Phase 2a Study of HU6 in Subjects with Elevated Liver Fat and High BMI Volunteers

Clinical Study Protocol

Version Number: 3.0

Date: 21 Dec 2021

Confidentiality Statement:

This document has been approved for publication by Rivus Pharmaceuticals, Inc.

SIGNATURE PAGE FOR SPONSOR

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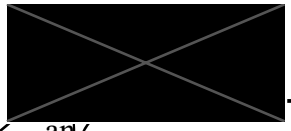
Approved by the following:



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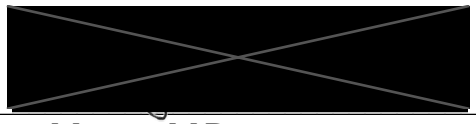

12-21-2021
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SIGNATURE PAGE FOR INVESTIGATOR

Study No. RIV-HU6-203

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I have read this protocol and agree to conduct this study in accordance with all stipulations of the protocol and in accordance with all applicable regulations, ICH and the Declaration of Helsinki.

Investigator Name	Signature	Date

1 STUDY ORGANIZATIONAL STRUCTURE

Sponsor:	Rivus Pharmaceuticals, Inc.
Primary Sponsor Contact:	Shaharyar Khan, Ph.D. Chief Scientific Officer Rivus Pharmaceuticals, Inc. 706b Forest Street Charlottesville, VA 22903 [redacted]
Clinical Project Manager	Catherine Ross MacLaren Clinical Project Manager [redacted]
24-Hour Medical Monitor Coverage	Roger Morgan, M.D. Vice President Medical Affairs MedSurgPI, LLC 3700 Lark Farm Road Franklinton, NC 27525 [redacted]

2 PROTOCOL SYNOPSIS

TITLE: A 61-day Randomized, Double blind, Placebo-controlled Trial to Assess the Safety and Efficacy of Three Doses of HU6 in Subjects with Elevated Liver Fat and High Body Mass Index (28.0 to 45.0 kg/m^2)
SPONSOR: Rivus Pharmaceuticals, Inc.
PROTOCOL NUMBER: RIV-HU6-203
CLINICAL STUDY PHASE: Phase 2a
INVESTIGATIONAL DRUG PRODUCT: HU6
Name of Active Ingredient (IUPAC name): [redacted]
No of Study Center(s): Up to 5 Centers
Study Period: Estimated date first subject enrolled: May 2021 Estimated last subject completed: September 2021
Study Duration per subject: Approximately 120 days (up to 45 days for screening, 61 days of dosing, up to 14 days for final visit after dosing completion)
Primary Objectives: **Efficacy**: To evaluate the reduction in liver fat content, as assessed by magnetic resonance imaging proton density fat fraction (MRI-PDFF) from baseline to Day 61 in subjects with elevated Body Mass Index (BMI) treated with HU6 compared to placebo. **Safety**: To evaluate safety and tolerability of 61 days of repeated daily dosing of HU6 in overweight and obese subjects as defined by BMI. **Secondary Objectives:** • To assess the rate and amount of body weight loss after 61 days of HU6 treatment. • To assess change from baseline in whole body adiposity by MRI after 61 days of HU6 treatment. • To characterize the pharmacokinetic (PK) profile of HU6 and its metabolites, 2,4-dinitrophenol (DNP) and M1, over 61 days of dosing in subjects with high BMI. • To evaluate and correlate changes from baseline in measures of liver composition with changes in liver fat content after dosing with HU6.

- To investigate the pharmacodynamic (PD) effects of HU6 on metabolic and cardiovascular risk factors.
- To investigate the PD effects of HU6 on metabolomic, proteomic, and lipidomic profiles.
- To characterize the dose/exposure relationships of the efficacy and PD effects of HU6, as data allow.

Endpoints:

Primary Endpoint:

- Relative change from baseline in liver fat content, as assessed MRI-PDFF at Day 61.

Secondary Endpoints:

Pharmacodynamics

- Change from baseline in body weight at Day 61.
- Change from baseline in whole body adiposity at Day 61.
- Changes from baseline in surrogate measures of liver inflammation and fibrosis Day 61: Fibroscan® Vibration-controlled Transient Elastography (VCTE), Fibroscan® Controlled Attenuation Parameter (CAP) score, and Enhanced Liver Fibrosis (ELF) score.
- Change from baseline in lipid parameters and cardiovascular risk biomarkers Day 61: serum high sensitivity C-reactive protein (hs-CRP), Lp(A), Apo B, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol, triglycerides, and free fatty acids (FFA).
- Change from baseline in metabolic disease parameters at Day 61: homeostatic model assessment for insulin resistance (HOMA-IR), fasting blood glucose concentrations, glycated albumin concentrations, and glycosylated hemoglobin (hemoglobin A_{1c} [HbA_{1c}].

Safety and Tolerability

- Summary of physical examinations findings over the course of the study.
- Adverse event (AE) assessment over the course of the study.
- Assessment of vital sign parameters over the course of the study. Parameters to include resting systolic and diastolic blood pressures, resting heart rates, resting respiratory rates, and oral body temperatures.
- Assessment of body weight over the course of the study.
- Safety 12-lead electrocardiograms (ECGs) over the course of the study.
- Assessment of clinical laboratory values (hematology, full biochemistry panel (including lipid panel, creatine phosphokinase [CPK], magnesium, liver function tests) and urinalysis (UA) over 61 days of dosing.

- Assessment of ophthalmologic examination, including slit lamp, prior to and after 61 days of dosing.

Pharmacokinetics

- Population PK (PPK) analysis of HU6 parent and metabolites, DNP and M1. The following PK parameters will be calculated: C_{max}, T_{max}, $t_{½}$, T_{lag}, AUC_{0-t}, $AUC_{0-\infty}$, CL/F, Vd/F, λ_z. Other PK parameters may be calculated, as data allow and appropriate.
- As data permit, noncompartmental analysis of HU6 parent and metabolites, DNP and M1. The following PK parameters will be calculated: C_{max}, AUC, and accumulation.
- Modeling of exposure response relationships of HU6, DNP and M1 and efficacy/PD endpoints, as appropriate.

Exploratory Endpoints:

- Change from baseline in metabolomic and lipidomic profiles (One Way Liver- [OWL] metabolomic and lipidomic assays) at Day 61.
- Change from baseline in proteomic profiles (SomaScan®) at Day 61.
- Change from baseline in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations at Day 61, as data allow.

NUMBER OF SUBJECTS:

Assuming 15% drop-out rate, approximately 80 subjects will be enrolled to achieve 68 evaluable subjects.

Eligible subjects will be randomized equally to 1 of the 4 following treatment groups (approximately 20 subjects per group):

- Group 1: matched placebo orally once daily for 61 days.
- Group 2: 150 mg HU6 orally once daily for 61 days.
- Group 3: 300 mg HU6 orally once daily for 61 days.
- Group 4: 450 mg HU6 orally once daily for 61 days.

The randomization will be blocked and stratified by HbA_{1c} (normal range versus between 5.7% and 9.0% inclusive).

Inclusion Criteria: Subjects must meet all the following inclusion criteria to be eligible:

1. Adult male or females, 28 to 65 years of age (inclusive) at the time of informed consent with BMI between 28.0 and 45.0 kg/m^2 (inclusive).
    a. Female subjects of childbearing potential must be non-lactating, not pregnant as confirmed by a negative urine pregnancy test at Screening and agree to continue using an effective method of contraception for at least 4 weeks or barrier method for 2 weeks prior to first study drug administration until 30 days after the last dose of study drug.

b. Female subjects of childbearing potential must not donate ova during the study and for at least 30 days after the last dose of study drug.

c. Female subjects of non-childbearing potential must be surgically sterile (e.g., hysterectomy, bilateral tubal ligation, oophorectomy) or postmenopausal (no menses for >1 year with follicle stimulating hormone (FSH) >40 U/L at Screening).

d. Male subjects who have not had a vasectomy and/or subjects who have had a vasectomy but have not had 2 post surgery negative tests for sperm must agree to use an acceptable method of contraception from time of first dose of study drug until 30 days after the last dose of the study drug, and to not donate sperm during the study and for at least 30 days after the last dose of study drug.

2. Inclusion as per investigator assessment of general medical status and as documented by medical history, physical examination, vital sign assessments, 12-lead ECG, clinical laboratory assessments, and general observations.

    a. Subjects must be on stable doses of medications for underlying obesity-related conditions for at least 2 months prior to screening.

    b. Subjects with diabetes may be treated with metformin, DPP-4 inhibitors, or sulfonylureas, but must be on stable doses for at least 2 months prior to screening.

    c. At Screening, certain laboratory values may be outside the reference range if commensurate with the underlying obesity or associated metabolic dysfunction in the eligible subject (for example, dyslipidemia and hyperglycemia), u nless these abnormalities suggest an underlying condition which may impact subject safety in the trial or interfere with the evaluation of HU6 or affect interpretation of the study results.

    d. Abnormalities or deviations outside the normal ranges for other assessments that are considered clinically significant by the Investigator (clinical laboratory tests, ECG, vital signs, physical examination) may be repeated once at the discretion of the Investigator(s). Results that continue to be outside the normal ranges must be judged by the investigator to be not clinically significant and acceptable for study participation.

    e. Subjects with elevation of unconjugated bilirubin due to presumptive Gilbert's syndrome are permissible.

    f. Subject must be euthyroid as assessed by a thyroid profile utilizing thyroid stimulating hormone (TSH) and free thyroxine (T4) testing at screening. Subjects with a stable history of thyroid disease and who have been on stable doses of thyroid medications for a minimum of 4 months can be enrolled.

3. Fibroscan® CAP score>300 dB/m. If the score is between >270 to 300 dB/m, it may be approved after discussions between the PI and Medical Monitor.

4. ≥8% liver fat by MRI-PDFF.

5. Understands the procedures and requirements of the study and provides written informed consent and authorization for protected health information disclosure.
6. Willing and able to comply with the requirements of the study protocol.

Exclusion Criteria: Subjects will be excluded from the study if any of the following criteria are met:

1. Insulin-controlled diabetes.
2. Pregnant or breastfeeding or plans to become pregnant.
3. Intolerance to Magnetic Resonance Imaging (MRI) or with conditions contraindicated for MRI procedures including but not limited to inability to fit into MRI scanner or surgical clips/metallic implants/shrapnel. Subjects must not be claustrophobic, have a history of claustrophobia, or intolerance of closed or small spaces.
4. Weight gain or loss >5% in 3 months prior to study or >10% in 6 months prior to screening.
5. History of lap banding, intragastric balloon, duodenal-jejunal sleeve, or bariatric surgery within 5 years of screening, plans for bariatric surgery prior to conclusion of study participation, or plans to lose weight during this study either through a special diet, exercise program or *both.* Subjects currently maintained on a special diet or exercise program must be willing to discontinue that program for 30 days prior to Day 1 and throughout the duration of the study.
6. History of malignant hyperthermia.
7. History of chronic serious recurrent skin rashes of unknown cause.
8. History of or current clinically significant cardiovascular disease including but not limited to transient ischemic attack, stroke, cardiac arrhythmias, syncope, unstable angina, myocardial infarction in the 6 months prior to screening, congestive heart failure, or uncontrolled hypertension. (Uncontrolled hypertension is defined as a systolic blood pressure ≥160 mmHg or a diastolic blood pressure ≥100 mmHg)
9. Resting heart rate <45 or >110 bpm.
10. On screening ECG or by history:
    a. A marked baseline prolongation of QT/QTcF interval (e.g., repeated demonstration of a QTcF interval > 450 msec for males and >470 msec for females).
    b. A history of additional risk factors for *Torsades de Pointes* (TdP) (e.g., heart failure, hypokalemia, family history of Long QT Syndrome) or a family history of sudden cardiac death of unknown origin.
11. Kidney disease, kidney transplant or estimated glomerular filtration rate (eGFR) <50 mL/min/1.73 m^2 based on the CKD-EPI Creatinine Equation (NKF 2009; https://www.kidney.org/content/ckd-epi-creatinine-equation-2009).

12. Significant lung disease requiring chronic daily medication including chronic obstructive pulmonary disease (COPD), emphysema, pulmonary fibrosis, or asthma. Documented well-controlled asthma is allowed.
13. Untreated obesity hypoventilation syndrome (OHS) or obstructive sleep apnea (OSA).
14. History of or active (acute or chronic) liver disease other than nonalcoholic fatty liver disease (NAFLD)/ nonalcoholic steatohepatitis (NASH), such as but not limited to autoimmune liver disease, viral hepatitis, genetic hemochromatosis, primary biliary cirrhosis, Wilson disease, alpha-1-antitrypsin deficiency, alcohol liver disease, acute fatty liver of pregnancy or drug- induced (including acetaminophen) liver disease.
15. History of or treatment for clinically significant gastroparesis, inflammatory bowel disease, or any surgery of the upper gastrointestinal tract with the exception of cholecystectomy, or minor gastric procedures that are approved by the medical monitor.
16. History of cirrhosis and/or hepatic decompensation, including ascites, hepatic encephalopathy, or variceal bleeding.
17. History of acute pancreatitis within one year of screening or chronic pancreatitis of any cause.
18. Serum triglyceride concentrations exceeding 500 mg/dL.
19. HbA_{1c} >9.0%.
20. Familial (mother/father/sibling) and/or personal history of spontaneous retinal detachment any time in the past.
21. Any history of or current diagnosis of Glaucoma.
22. Evidence of the following pathologies on screening ophthalmologic examination:
    a. Peripheral retinal pathology requiring treatment, retinal tears, or lattice that require treatment.
    b. Diabetic retinopathy with macula exudates or macula edema as shown by optical coherence tomography (OCT) and examination.
    c. Any active macular disease that affects the vision, including macula pucker (epiretinal membrane) and macular degeneration.
    d. Visually significant cataract as determined by ophthalmologist.
    e. Any previous intravitreal injection of anti-VEGF agents for macular degeneration.
    f. History of prior vitrectomy.
23. History of malignant neoplasms within 5 years of screening, except for basal cell or squamous cell skin cancer, cervical carcinoma *in situ*, or prostate cancer that is not

currently or expected to require radiation therapy, chemotherapy and/or surgical interventions or to initiate hormonal treatment.

24. History of organ transplantation.
25. Received a COVID-19 vaccine less than 1 week prior to dosing (Visit 2 / Day 1) and/or plans to receive a COVID-19 vaccine during the study period.
26. History of significant drug abuse within one year prior to Screening or frequent use of soft drugs (such as marijuana) within 3 months prior to the Screening visit, or hard drugs (such as cocaine, phencyclidine [PCP], opioid derivatives including heroin, and amphetamine derivatives) within 1 year prior to screening.
27. History of alcoholism in the last 2 years or current evidence of excessive alcohol consumption as assessed by screening evaluation using the Alcohol Use Disorders Identification Test (AUDIT, Thompson 2018 [Appendix A]), and history of regular alcohol consumption exceeding approximately14 drinks/week for men and 7 drinks/week for women [1 drink = 4 ounces (120 mL) of wine or 12 ounces (360 mL) of beer or 1 ounce (30 mL) of hard liquor] within 6 months of Screening, as determined by the Investigator.
28. Positive urine drug screen for drugs of abuse or positive phosphatidylethanol (PEth) blood test result >200 ng/mL based on tPEth 16.0/18.1 (POPEth) at Screening. In instances of an exclusionary PEth value, consideration for enrollment can be provided if the principal investigator and medical monitor agree the subject's history is not consistent with alcohol abuse. Subjects with a positive drug screen due to an approved medication may be allowed on a case-by-case basis by the Investigator in consultation with the medical monitor.
29. Current regular vaping or more than 10 cigarettes or the equivalent per week. Use of nicotine patches for smoking cessation is permitted.
30. Positive test results of hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV Ab), or human immunodeficiency virus (HIV1/2) antibody.
31. Neutropenia, defined as absolute neutrophil count ≤1000/μL.
32. Serum AST or ALT >5 x upper limit of normal (ULN) at screening. (One repeat test may be allowed within 7 days at the discretion of the Investigator).
33. Total bilirubin > ULN, unless due to Gilbert's syndrome or if considered normal variability in the absence of other clinically relevant liver impairment, as approved by the medical monitor.
34. International normalized ratio (INR) ≥1.3 at screening if there is other evidence of potentially significant liver impairment.
35. Participation in another clinical trial at the time of screening or exposure to any investigational agent, including topical, within 30 days of screening or 5 half-lives, if half-life known.

36. No tattoo or body piercings during the course of the study.
37. Any underlying physical or psychological medical condition that, in the opinion of the Investigator or sponsor, would make it unlikely that the subject is able comply with the study requirements or would be unable to complete the study.
38. Any condition that the investigator believes would interfere with his/her ability to provide written informed consent, comply with study instructions, or which might confound the interpretation of the study results or put the subject at undue risk.
39. Known or potential hypersensitivity to HU6 or its excipients.

Prohibited Medications (Current Use):

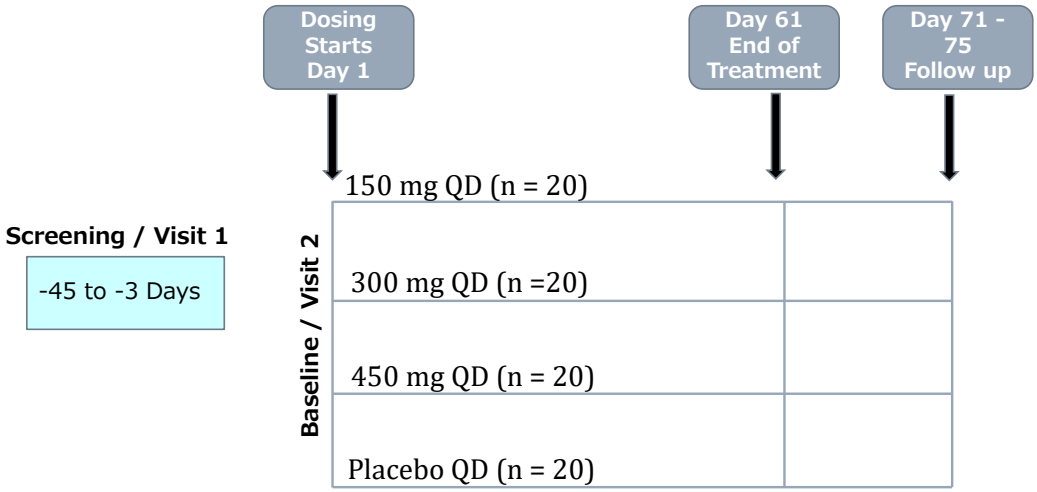
40. Any herbal supplement, over the counter drug, mail order or prescription drug for weight loss.
41. Prescription or over the counter stimulants including: dextroamphetamine/Dexedrine, dextroamphetamine/amphetamine combination product/Adderall, or methylphenidate (Ritalin®, Concerta®).
42. Thiazolidinediones (TZD): pioglitazone/Actos, rosiglitazone/Avandia.
43. Glucagon-like peptide 1 (GLP1) agonists: exenatide/Byetta/Bydureon, lixisenatide/Adlyxin, liraglutide/Victoza, dulaglutide/Trulicity, semaglutide/Ozempic.
44. Sodium-glucose cotransporter-2 (SGLT2) inhibitors: canagliflozin/Invokana, dapagliflozin/Farxiga, empagliflozin/Jardiance, ertugliflozin/Steglatro.
45. Vitamin E: use of ursodiol or high dose vitamin E >400 IU/day for at least one month within in the last 6 months or started high dose vitamin E within last 3 months of screening.
46. Recent (within 3 months of screening) or current use of obeticholic acid/Ocaliva, systemic corticosteroids, methotrexate, tamoxifen, amiodarone, or long-term use of tetracyclines.
47. Warfarin, heparin, factor Xa inhibitors (dabigatran, betrixaban, edoxaban, apixaban, and rivaroxaban).
48. Concomitant medications that prolong the QT/QTc interval and are known to be associated with increased risk of Torsade des pointes as identified in the https://crediblemeds.org/ website list category of '**Known Risk'** (Appendix B). For subjects on medications that are listed in Appendix B, approval must be obtained from the Sponsor and Medical Monitor.

METHODOLOGY:

Overview of Study Design

Subjects will be screened over a 45 day period to determine their eligibility based on specific history, physical, laboratory and imaging evaluations as per the Schedule of Assessments. Due to scheduling of the procedures, multiple visits will likely be necessary to complete the screening process. However, if all screening assessments and procedures can be completed within 30 days of first dose that is permissible.

Once qualified, patients will be randomly assigned to one of the HU6 treatment groups or the matched placebo control group and dosed once daily (fasting) for a total of 61 days. Subjects will return to the clinic for frequent assessment visits during the 61 days of dosing as demonstrated in the Schedule of Assessments (Section 8.1). A follow-up visit will occur within 10-14 days following the completion of dosing.



10 Visits, Screening, Baseline/ First dose, Day 2, Day 7, Day 14, Day 28, Day 42, Day 49, Day 61 / end of treatment, and Follow up at Day 71 – 75.

Visit procedures include PK and PD measures and safety assessments.

Subjects will be instructed to maintain their same diet and activity/exercise level throughout the study as they had prior to participation in the study.

DOSAGE, DOSE FORM, AND ROUTE OF ADMINISTRATION:

Study drug will be supplied to the clinic as bulk capsules in a polyethylene bag contained in high-density polyethylene (HDPE) secondary container. The study drug is prepared by filling 150 mg of HU6 in a size 00 HPMC capsule. Bulk drug product should be stored at controlled room temperature in the original container.

Doses will be dispensed to each subject during dispensation visits in a 'pill organizer' with the appropriate pharmacy label that will include the subject's study number. The pill organizers containing the study drug should be stored at controlled room temperature, away from direct light.

Bulk matching placebo capsules (each size 00 HPMC capsule containing 150 mg of mannitol) will be supplied. The unblinded pharmacist will prepare placebo capsules in the same method as for the active study product.

HU6 will be administered orally in the fasted state (after a minimum 8-hour overnight fast).

STATISTICAL METHODS:

Size and power assumptions

A sample size of 17 subjects per group provides at least 80% power to detect a 30% difference in the mean relative change from baseline in liver fat content at Day 61, when the standard deviation is 30% [PASS 2020: Two sample t-test, alpha=.05]. Assuming a 15% dropout rate, up to 80 subjects should be enrolled to ensure that at least 68 subjects, 17 per group, are evaluable for the primary efficacy endpoint analysis.

Statistical Methods

Descriptive statistical methods will be used to summarize the data from this study, with statistical testing performed for the efficacy endpoints. Unless stated otherwise, the term "descriptive statistics" refers to number of subjects (n), mean, median, standard deviation, minimum, and maximum for continuous data, and frequencies and percentages for categorical data. Statistical testing, if performed, will be 2-sided and will be performed using a significance (alpha) level of 0.05. There will be no adjustment for multiple comparisons for this exploratory study. All available data for enrolled subjects will be listed by subject. Unless otherwise noted, the data will be sorted first by subject number and then by date within each subject number. All statistical analyses will be conducted with the SAS® System, version 9.4 or higher.

TABLE OF CONTENTS

GLOSSARY OF TERMS AND ABBREVIATIONS

Abbreviation	Description
AE	Adverse event
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AUC	Area under the concentration – time curve
AUDIT	Alcohol Use Disorders Identification Test
BMI	Body mass index (kg/m^2)
BUN	Blood urea nitrogen
C	Celsius
CAP	Controlled attenuation parameter score
CBD	Cannabidiol
CI	Confidence interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
C_{max}	Maximum observed plasma drug concentration
C_{ss}	Steady state concentration
COPD	Chronic obstructive pulmonary disease
CPK	Creatine phosphokinase
CRA	Clinical research associate
CV	Coefficient of variation
DILI	Drug induced liver injury
dL	Deciliter
DNP	2,4-dinitrophenol
ECG	Electrocardiogram
eCRFs	Electronic case report forms
eGFR	Estimated glomerular filtration rate
ELF	Enhanced liver fibrosis
F	Fahrenheit
FAS	Full analysis set

Abbreviation	Description
FFA	Free fatty acid
FSH	Follicle stimulating hormone
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
GLP	Good Laboratory Practices
GLP1	Glucagon-like peptide 1
h	Hour(s)
HbA_{1c}	Glycosylated hemoglobin (hemoglobin A1c)
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HCVab	Hepatitis C virus antibody
HDL	High-density lipoprotein
HDPE	High-density polyethylene
HIV	Human immunodeficiency virus
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HPMC	Hydroxypropyl methylcellulose
hs-CRP	High sensitivity C-reactive protein
IC	Indirect calorimetry
ICF	Informed consent form
ICH	International Council for Harmonisation
INR	International normalized ratio
IRB	Institutional Review Board
ITT	Intent-to-treat
IV	Intravenous
kg	Kilogram
LFTs	Liver function tests
LDL	Low-density lipoprotein
LSM	Least square mean
MAD	Multiple ascending dose
MCHC	Mean corpuscular hemoglobin concentration

Abbreviation	Description
MCV	Mean cell volume
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed model for repeated measures
MRI	Magnetic resonance imaging
MRI-PDFF	Magnetic resonance imaging proton density fat fraction
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
OCT	Optical coherence tomography
OHS	Obesity hypoventilation syndrome
OSA	Obstructive sleep apnea
OWL	One Way Liver
PCP	Phencyclidine
PD	Pharmacodynamic(s)
PEth	Phosphatidylethanol
PIIINP	Procollagen III amino-terminal peptide
PK	Pharmacokinetic(s)
PPK	Population pharmacokinetic(s)
QD	Once daily
QTcF	QT interval corrected for heart rate using Fridericia cubed root formula
RMR	Resting metabolic rate
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SGLT2	Sodium-glucose cotransporter-2
SOC	System organ class
SomaScan	Slow off-rate modified aptamer
T4	Thyroxine
TdP	Torsades de pointes
TIMP-1	Tissue inhibitor of matrix metalloproteinase 1
T_{max}	Time to peak plasma concentration
TSH	Thyroid stimulating hormone

Abbreviation	Description
TZD	Thiazolidinediones
UA	Urinalysis
ULN	Upper limit of normal
VCTE	Vibration-controlled transient elastography
VLDL	Very low-density lipoproteins
WHO	World Health Organization

3 INTRODUCTION

Omitted.

4 STUDY OBJECTIVES

4.1 Primary Objectives

Efficacy:

- To evaluate the reduction in liver fat content, as assessed by magnetic resonance imaging proton density fat fraction (MRI-PDFF) from baseline to Day 61 in subjects with elevated BMI treated with HU6 compared to placebo.

Safety:

- To evaluate safety and tolerability of 61 days of repeated daily dosing of HU6 in overweight and obese subjects as defined by BMI.

4.2 Secondary Objectives

- To assess the rate and amount of body weight loss after 61 days of HU6 treatment.
- To assess change from baseline in whole body adiposity by MRI after 61 days of HU6 treatment.
- To characterize the PK profile of HU6 and its metabolites, DNP and M1, over 61 days of dosing in subjects with high BMI.
- To evaluate and correlate changes from baseline in measures of liver composition with changes in liver fat content after dosing with HU6.
- To investigate the pharmacodynamic (PD) effects of HU6 on metabolic and cardiovascular risk factors.
- To investigate the PD effects of HU6 on metabolomic, proteomic, and lipidomic profiles.
- To characterize the dose/exposure relationships of the efficacy and PD effects of HU6, as data allow.

5 STUDY TYPE AND DESIGN

5.1 Study Type

This is a Phase 2a, randomized, parallel-group, placebo-controlled, double-blind, repeated-dose study.

5.2 Endpoints

5.2.1 Primary Endpoint

- Relative change from baseline in liver fat content, as assessed by MRI-PDFF at Day 61.

5.2.2 Secondary Endpoints

Pharmacodynamics

- Change from baseline in body weight at Day 61.
- Change from baseline in whole body adiposity at Day 61.
- Changes from baseline in surrogate measures of liver inflammation and fibrosis at Day 61: Fibroscan® Vibration-controlled Transient Elastography (VCTE), Fibroscan® Controlled Attenuation Parameter (CAP) score, and Enhanced Liver Fibrosis (ELF) score.
- Change from baseline in lipid parameters and cardiovascular risk biomarkers at Day 61: serum high sensitivity C-reactive protein (hs-CRP), Lp(A), Apo B, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol, triglycerides, and free fatty acids (FFA).
- Change from baseline in metabolic disease parameters at Day 61: homeostatic model assessment for insulin resistance (HOMA-IR), fasting blood glucose concentrations, glycated albumin concentrations, HbA_{1c}.

Safety and Tolerability

- Summary of physical examinations findings over the course of the study.
- Adverse event (AE) assessment over the course of the study.
- Assessment of vital sign parameters over the course of the study. Parameters to include resting systolic and diastolic blood pressures, resting heart rates, resting respiratory rates, and oral body temperatures.
- Assessment of body weight over the course of the study.
- Safety 12-lead ECGs over the course of the study.
- Assessment of clinical laboratory values (hematology, full biochemistry panel (including lipid panel, CPK, magnesium, liver function tests) and urinalysis (UA) over 61 days of dosing.
- Assessment of ophthalmologic examination, including slit lamp, prior to and after 61 days of dosing.

Pharmacokinetics

- Population PK analysis of HU6 parent and metabolites, DNP and M1. The following PK parameters will be estimated as appropriate: C_{max}, T_{max}, $t_{½}$, T_{lag}, AUC_{0-t}, $AUC_{0-\infty}$, CL/F, Vd/F, λ_z. Other PK parameters may be calculated, as data allow and appropriate.
- As data permit, noncompartmental analysis of HU6 parent and metabolites, DNP and M1. The following PK parameters will be calculated: C_{max}, AUC, and accumulation.
- Modeling of exposure response relationships of HU6, DNP and M1 and efficacy/pharmacodynamic endpoints, as appropriate.

5.2.3 Exploratory Endpoints

- Change from baseline in metabolomic and lipidomic profiles (One Way Liver-[OWL] metabolomic and lipidomic assays) at Day 61.
- Change from baseline in proteomic profiles (SomaScan®) at Day 61.
- Change from baseline in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations at Day 61, as data allow.

5.3 Study Design

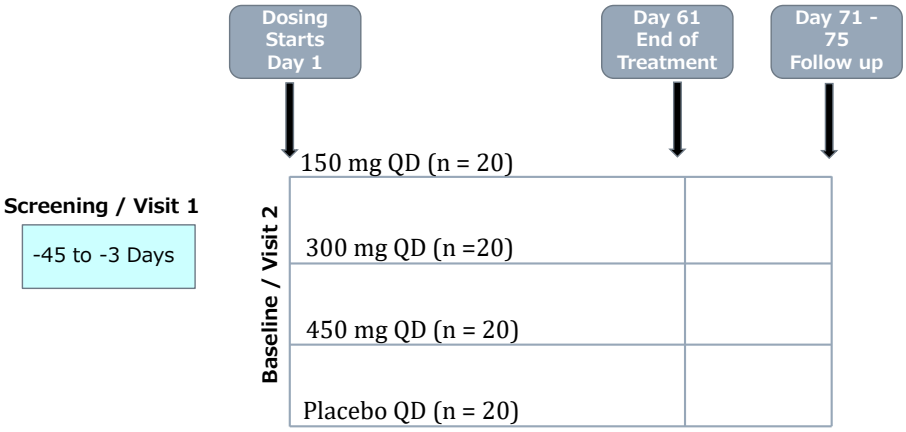
This is a Phase 2a, randomized, parallel-group, placebo-controlled, double-blind, repeated-dose study to evaluate the safety and efficacy of three oral dose levels of HU6 compared to placebo over the course of 61 days in subjects with high BMI and evidence of elevated liver fat.

Subjects will be screened over a 45 day period to determine their eligibility based on specific history, physical, laboratory and imaging evaluations as per the Schedule of Assessments (Section 8.1). Due to scheduling of the procedures, multiple visits will likely be necessary to complete the screening process. However, if all screening assessments and procedures, including the MRI, can be completed within 30 days of the first dose, then a single screening visit is permissible (see Section 8.2.1).

Once qualified, patients will be randomly assigned to one of the HU6 treatment groups or the matched placebo control group and dosed once daily (fasting) for a total of 61 days. Subjects will return to the clinic for frequent assessment visits during the 61 days of dosing. A follow-up visit will occur within 10 to 14 days following the completion of dosing (

Figure 1).

Figure 1: Study Schematic



10 Visits, Screening, Baseline/ First dose, Day 2, Day 7, Day 14, Day 28, Day 42, Day 49, Day 61 / end of treatment, and Follow up at Day 71 – 75.

Visit procedures include PK and PD measures and safety assessments.

Subjects will be instructed to maintain their same diet and activity/exercise level throughout the study as they had prior to participation in the study.

Dosing will take place once daily in a fasted state. Eligible subjects will be randomized equally (N = 20 per group) to 1 of 4 treatment groups:

- Group 1: matched placebo orally once daily for 61 days.
- Group 2: 150 mg HU6 orally once daily for 61 days.
- Group 3: 300 mg HU6 orally once daily for 61 days.
- Group 4: 450 mg HU6 orally once daily for 61 days.

The randomization will be blocked and stratified by HbA_{1c} (normal range versus between 5.7% and 9.0% inclusive).

5.4 Study Duration

For an individual subject, the study will last approximately 120 days (up to 45 days for screening, 61 days of dosing, up to 14 days for final visit after dosing completion.

The entire study is estimated to last approximately 5 months: first subject in May 2021 with last subject anticipated being completed in September 2021.

6 STUDY DRUG

6.1 Supply and Storage

Study drug will be supplied to the clinic as bulk capsules in a polyethylene bag contained in high-density polyethylene (HDPE) secondary container. The study drug is prepared by filling 150 mg of HU6 in a size 00 HPMC capsule. Bulk drug product should be stored at controlled room temperature in the original container.

Doses will be dispensed to each subject during dispensation visits in a 'pill organizer' with the appropriate pharmacy label that will include the subject's study number. The pill organizers containing the study drug should be stored at controlled room temperature, away from direct light.

Bulk matching placebo capsules (each size 00 HPMC capsule containing 150 mg of mannitol) will be supplied. The unblinded pharmacist will prepare placebo capsules in the same method as for the active study product.

The unblinded pharmacist will prepare study drug such that the double-blind is maintained.

Refer to the Pharmacy Manual for more information about study drug preparation and dispensing.

6.2 Administration

6.2.1 Dosing Instructions

At Visit 2, clinical personnel will train subjects on dosing procedures. On Days 1-61, study drug will be administered once a daily in the morning after a minimum 8-hour overnight fast.

Study drug will be administered in-clinic during each on-treatment study visit (Visits 2 through 9). Subjects should not take their dose of study drug at home on these mornings. Subjects should present to the clinic in the fasted state. Predose assessments will be conducted, and subjects will be dosed by clinical personnel during the on-treatment study visits. Subjects will remain fasted (from food and drink other than water) for at least 1-hour postdose. Capsules will be swallowed with approximately 240 mL (8 fluid ounces) of room temperature water.

On all other dosing days, subjects will take their assigned dose of study drug at home in the fasted state (minimum 8-hour fast), and subjects should remain fasted from food until at least 1-hour postdose. Clear liquids are permitted.

Study drug will be dispensed at Study Visits 3 through 8. Each subject will receive supply of study drug at each dispensing visit to be used until the next visit. Refer to the Study Procedures Manual for more information about study drug dispensing.

6.2.2 Daily Dosing Log

On each dosing day, subjects will be asked to complete a daily dosing log. Subjects will be instructed in the use of a thermometer to document body temperature, and subjects will be trained on daily completion of the log. Subjects will complete the log on each dosing day to document daily dosing, body temperature, and meal intake. Subjects will bring the completed dosing log along and their thermometer

with them to their study visits. The clinic staff should review the data in the log with the subject. The thermometer also will have recorded temperatures for the preceding 9 days and should be reviewed by the study coordinator with the subject.

The log will ask for information such as:

- Date
- Did you take your dose of study drug this morning? (Yes/No)
- What is your body temperature?
- In the last 24 hours prior to this morning's dose, did you eat Less/Same/More than you normally do?

The purpose of the Daily Log is help ensure and reinforce compliance to subject instructions as a result of the review and discussion with the study coordinator. Individual results are not to be entered into the case record form (CRF). An AE that is identified by the review and discussion of the log with the subject should be documented appropriately (Section 11.7).

6.2.3 Compliance

Subjects will be instructed to bring any used (empty bottles), partially used, or unopened bottles to each study visit. During the study, the clinic staff will monitor adherence by counting capsules at each visit and by evaluating the daily dosing log. Clinic staff will reinforce compliance with study procedures and troubleshoot any problem that may be influencing treatment adherence. Should there be consistently poor compliance with dosing, despite attempts to help the subject comply, there should be a discussion with the medical monitor to determine next steps.

6.3 Packaging and Labeling

Study drug will be supplied to the clinic as bulk powder in a polyethylene bag contained in an HDPE container.

- The label on the bulk product will contain the following information in the English language:
- Protocol number: RIV-HU6-203
- Expiration date
- Lot number
- Contents
- Weight
- Storage conditions
- The sentence, Caution: New Drug Limited by Federal (US) Law to Investigational Use
- Name of the Investigator
- Name, address, and telephone number of the Sponsor.

6.4 Accountability

The Investigator or their appointed designee is responsible for ensuring that deliveries of study drug are correctly dispensed and recorded, that the product is handled and stored safely and properly, and that it is only being given to subjects in accordance with this protocol.

Sites will keep a current log of drug accountability recording:

- Study drug supply received from the Sponsor.
- Study drug supply dispensed to each subject.
- Study drug supply currently in inventory.
- Study drug supply destroyed or returned to the Sponsor (or third party) for destruction.

Note: Drug accountability is the responsibility of the Investigator; a written account will be required for all discrepancies.

The Sponsor's designated Monitor must verify all accountability records during periodic monitoring visits. Unused and used study drug must be stored on site until such accountability has taken place and authorization is received from the Sponsor or Sponsor's designee that the study drug may be returned or destroyed.

6.5 Overdose/Toxicity Management

No specific pharmacologic antagonist or antidote exists for HU6. Therefore, overdose or clinical toxicity should be managed with supportive care and pharmacologic treatments directed at specific symptoms (i.e. benzodiazepines for agitation or antipyretics for fever.)

Should an overdose occur in the clinic which may impose on the subject significantly higher pharmacologic levels than have previously been studied, the subject (at the discretion of the PI or medical monitor) may be more intensively monitoring, including but not limited to continuous telemetry. Should prolongation of QTc interval be seen in excess of 500 msec, the subject should be placed on continuous telemetry until the interval returns to less than or equal to 500 msec.

6.6 Blinding and Randomization

This is a double-blind, placebo-controlled study. The subjects, clinical personnel, and Sponsor personnel will be blinded. Only in the case of an emergency, when knowledge of the investigational product is essential for the clinical management or welfare of the subject, may the investigator unblind a subject's treatment assignment.

The investigator will, whenever possible, discuss options with the Medical Monitor, or appropriate Sponsor study personnel before unblinding. If the blind is broken for any reason before the investigator is able to contact the Medical Monitor or Sponsor, the investigator must notify the Medical Monitor or Sponsor as soon as possible following the unblinding incident without revealing the subject's study treatment assignment, unless the information is important to the safety of subjects remaining in the study. In addition, the investigator will record the date, time and reason for revealing the blinded treatment assignment for that subject.

If a serious adverse event (SAE; as defined in Section 11.8.1) is reported to the Medical Monitor or Sponsor and unblinding is agreed upon, the Investigator may unblind the treatment assignment for the individual subject. If an expedited regulatory report to one or more regulatory agencies is required, the report will identify the subject's treatment assignment. When applicable, a copy of the regulatory report may be sent to investigators in accordance with relevant regulations, Sponsor policy, or both.

Study drug will consist of HU6 and a matching placebo. The placebo will be identical in appearance to the active HU6. An unblinded pharmacist (or designee) at the study site will prepare medication before use according to instructions provided separately. The pharmacist will be required to maintain the blind, and the pharmacist will not otherwise participate in this study.

Treatment assignments will be based on a computer-generated randomization code provided by unblinded statistician. Sponsor personnel, including those involved in monitoring, data management, and data analysis, will not have access to the treatment codes during the trial. Access to the treatment codes will be restricted to personnel charged with generating and maintaining randomization files, preparing study drug, analyzing plasma concentrations, and reporting SAEs to regulatory agencies.

Once a randomization assignment has been made for a subject, it must not be reassigned.

7 STUDY POPULATION

7.1 Number of Subjects

Assuming 15% drop-out rate, approximately 80 subjects will be randomized to achieve 68 evaluable subjects.

7.2 Inclusion Criteria

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

1. Adult male or females, 28 to 65 years of age (inclusive) at the time of informed consent with BMI between 28.0 and 45.0 kg/m^2 (inclusive).
    a. Female subjects of childbearing potential must be non-lactating, not pregnant as confirmed by a negative urine pregnancy test at Screening and agree to continue using an effective method of contraception for at least 4 weeks or barrier method for 2 weeks prior to first study drug administration until 30 days after the last dose of study drug (Section 8.3.2).
    b. Female subjects of childbearing potential must not donate ova during the study and for at least 30 days after the last dose of study drug.
    c. Female subjects of non-childbearing potential must be surgically sterile (e.g., hysterectomy, bilateral tubal ligation, oophorectomy) or postmenopausal (no menses for >1 year with follicle stimulating hormone (FSH) >40 U/L at Screening).
    d. Male subjects who have not had a vasectomy and/or subjects who have had a vasectomy but have not had 2 post surgery negative tests for sperm must agree to use an acceptable method of contraception from time of first dose of study drug until 30 days after the last dose of the

study drug, and to not donate sperm during the study and for at least 30 days after the last dose of study drug.

2. Inclusion as per investigator assessment of general medical status and as documented by medical history, physical examination, vital sign assessments, 12-lead ECG, clinical laboratory assessments, and general observations.
    a. Subjects must be on stable doses of medications for underlying obesity-related conditions for at least 2 months prior to screening.
    b. Subjects with diabetes may be treated with metformin, DPP-4 inhibitors, or sulfonylureas, but must be on stable doses for at least 2 months prior to screening.
    c. At Screening, certain laboratory values may be outside the reference range if commensurate with the underlying obesity or associated metabolic dysfunction in the eligible subject (for example, dyslipidemia and hyperglycemia), unless these abnormalities suggest an underlying condition which may impact subject safety in the trial or interfere with the evaluation of HU6 or affect interpretation of the study results.
    d. Abnormalities or deviations outside the normal ranges for other assessments that are considered clinically significant by the Investigator (clinical laboratory tests, ECG, vital signs, physical examination) may be repeated once at the discretion of the Investigator(s). Results that continue to be outside the normal ranges must be judged by the investigator to be not clinically significant and acceptable for study participation.
    e. Subjects with elevation of unconjugated bilirubin due to presumptive Gilbert's syndrome are permissible.
    f. Subject must be euthyroid as assessed by a thyroid profile utilizing thyroid stimulating hormone (TSH) and free thyroxine (T4) testing at screening. Subjects with a stable history of thyroid disease and who have been on stable doses of thyroid medications for a minimum of 4 months can be enrolled.
3. Fibroscan® CAP score>300 dB/m. If the score is between >270 to 300 dB/m, it may be approved after discussions between the PI and Medical Monitor.
4. ≥8% liver fat by MRI-PDFF.
5. Understands the procedures and requirements of the study and provides written informed consent and authorization for protected health information disclosure.
6. Willing and able to comply with the requirements of the study protocol.

7.3 Exclusion Criteria

Subjects will be excluded from the study if any of the following criteria are met:

1. Insulin-controlled diabetes.
2. Pregnant or breastfeeding or plans to become pregnant.

3. Intolerance to Magnetic Resonance Imaging (MRI) or with conditions contraindicated for MRI procedures including but not limited to inability to fit into MRI scanner or surgical clips/metallic implants/shrapnel. Subjects must not be claustrophobic, have a history of claustrophobia, or intolerance of closed or small spaces.
4. Weight gain or loss >5% in 3 months prior to study or >10% in 6 months prior to screening.
5. History of lap banding, intragastric balloon, duodenal-jejunal sleeve, or bariatric surgery within 5 years of screening, plans for bariatric surgery prior to conclusion of study participation, or plans to lose weight during this study either through a special diet, exercise program or *both.* Subjects currently maintained on a special diet or exercise program must be willing to discontinue that program for 30 days prior to Day 1 and throughout the duration of the study.
6. History of malignant hyperthermia.
7. History of chronic serious recurrent skin rashes of unknown cause.
8. History of or current clinically significant cardiovascular disease including but not limited to transient ischemic attack, stroke, cardiac arrhythmias, syncope, unstable angina, myocardial infarction in the 6 months prior to screening, congestive heart failure, or uncontrolled hypertension. (Uncontrolled hypertension is defined as a systolic blood pressure ≥160 mmHg or a diastolic blood pressure ≥100 mmHg).
9. Resting heart rate <45 or >110 bpm.
10. On screening ECG or by history:
    a. A marked baseline prolongation of QT/QTcF interval (e.g., repeated demonstration of a QTcF interval > 450 msec for males and >470 msec for females).
    b. A history of additional risk factors for *Torsades de Pointes* (TdP) (e.g., heart failure, hypokalemia, family history of Long QT Syndrome) or a family history of sudden cardiac death of unknown origin.
11. Kidney disease, kidney transplant, or estimated glomerular filtration rate (eGFR) <50 mL/min/1.73 m^2 based on the CKD-EPI Creatinine Equation (NKF 2009; https://www.kidney.org/content/ckd-epi-creatinine-equation-2009).
12. Significant lung disease requiring chronic daily medication including chronic obstructive pulmonary disease (COPD), emphysema, pulmonary fibrosis, or asthma. Documented well-controlled asthma is allowed.
13. Untreated obesity hypoventilation syndrome (OHS) or obstructive sleep apnea (OSA).
14. History of or active (acute or chronic) liver disease other than nonalcoholic fatty liver disease (NAFLD)/ nonalcoholic steatohepatitis (NASH), such as but not limited to autoimmune liver disease, viral hepatitis, genetic hemochromatosis, primary biliary cirrhosis, Wilson disease, alpha-1-antitrypsin deficiency, alcohol liver disease, acute fatty liver of pregnancy or drug- induced (including acetaminophen) liver disease.

15. History of or treatment for clinically significant gastroparesis, inflammatory bowel disease, or any surgery of the upper gastrointestinal tract with the exception of cholecystectomy, or minor gastric procedures that are approved by the medical monitor.
16. History of cirrhosis and/or hepatic decompensation, including ascites, hepatic encephalopathy, or variceal bleeding.
17. History of acute pancreatitis within one year of screening or chronic pancreatitis of any cause.
18. Serum triglyceride concentrations exceeding 500 mg/dL.
19. HbA_{1c} >9.0%.
20. Familial (mother/father/sibling) and/or personal history of spontaneous retinal detachment any time in the past.
21. Any history of or current diagnosis of Glaucoma.
22. Evidence of the following on screening ophthalmologic examination:
    a. Peripheral retinal pathology requiring treatment, retinal tears, or lattice that require treatment.
    b. Diabetic retinopathy with macula exudates or macula edema as shown by optical coherence tomography (OCT) and examination.
    c. Any active macular disease that affects the vision, including macula pucker (epiretinal membrane) and macular degeneration.
    d. Visually significant cataract as determined by ophthalmologist.
    e. Any previous intravitreal injection of anti-VEGF agents for macular degeneration.
    f. History of prior vitrectomy.
23. History of malignant neoplasms within 5 years of screening, except for basal cell or squamous cell skin cancer, cervical carcinoma *in situ*, or prostate cancer that is not currently or expected to require radiation therapy, chemotherapy and/or surgical interventions or to initiate hormonal treatment.
24. History of organ transplantation.
25. Received a COVID-19 vaccine less than 1 week prior to dosing (Visit 2 / Day 1) and/or plans to receive a COVID-19 vaccine during the study period.
26. History of significant drug abuse within one year prior to Screening or frequent use of soft drugs (such as marijuana) within 3 months prior to the Screening visit, or hard drugs (such as cocaine, phencyclidine [PCP], opioid derivatives including heroin, and amphetamine derivatives) within 1 year prior to screening.
27. History of alcoholism in the last 2 years or current evidence of excessive alcohol consumption as assessed by screening evaluation using the Alcohol Use Disorders Identification Test (AUDIT, Thompson 2018 [Appendix A]), and history of regular alcohol consumption exceeding approximately 14 drinks/week for men and 7 drinks/week for women [1 drink = 4 ounces (120 mL)

of wine or 12 ounces (360 mL) of beer or 1 ounce (30 mL) of hard liquor] within 6 months of Screening, as determined by the Investigator.

28. Positive urine drug screen for drugs of abuse or positive phosphatidylethanol (PEth) blood test result >200 ng/mL based on tPEth 16.0/18.1 (POPEth) at Screening. In instances of an exclusionary PEth value, consideration for enrollment can be provided if the principal investigator and medical monitor agree the subject's history is not consistent with alcohol abuse. Subjects with a positive drug screen due to an approved medication may be allowed on a case-by-case basis by the Investigator in consultation with the medical monitor.
29. Current regular vaping or more than 10 cigarettes or the equivalent per week. Use of nicotine patches for smoking cessation is permitted.
30. Positive test results of hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV Ab), or human immunodeficiency virus (HIV1/2) antibody.
31. Neutropenia, defined as absolute neutrophil count ≤1000/μL.
32. Serum AST or ALT >5 x upper limit of normal (ULN) at screening. (One repeat test may be allowed within 7 days at the discretion of the Investigator).
33. Total bilirubin > ULN, unless due to Gilbert's syndrome or if considered normal variability in the absence of other clinically relevant liver impairment as approved by Medical Monitor.
34. International normalized ratio (INR) ≥1.3 at screening if there is other evidence of potential significant liver impairment.
35. Participation in another clinical trial at the time of screening or exposure to any investigational agent, including topical, within 30 days of screening or 5 half-lives, if half-life known.
36. No tattoo or body piercings during the course of the study. Any underlying physical or psychological medical condition that, in the opinion of the Investigator or sponsor, would make it unlikely that the subject is able comply with the study requirements or would be unable to complete the study.
37. Any condition that the investigator believes would interfere with his/her ability to provide written informed consent, comply with study instructions, or which might confound the interpretation of the study results or put the subject at undue risk.
38. Known or potential hypersensitivity to HU6 or its excipients.

Prohibited Medications (Current Use):

39. Any herbal supplement, over the counter drug, mail order or prescription drug for weight loss.
40. Prescription or over the counter stimulants including: dextroamphetamine/Dexedrine, dextroamphetamine/amphetamine combination product/Adderall, or methylphenidate (Ritalin®, Concerta®).
41. Thiazolidinediones (TZD): pioglitazone/Actos, rosiglitazone/Avandia.
42. Glucagon-like peptide 1 (GLP1) agonists: exenatide/Byetta/Bydureon, lixisenatide/Adlyxin, liraglutide/Victoza, dulaglutide/Trulicity, semaglutide/Ozempic.

43. Sodium-glucose cotransporter-2 (SGLT2) inhibitors: canagliflozin/Invokana, dapagliflozin/Farxiga, empagliflozin/Jardiance, ertugliflozin/Steglatro.
44. Vitamin E: use of ursodiol or high dose vitamin E >400 IU/day for at least one month within in the last 6 months or started high dose vitamin E within last 3 months of screening.
45. Recent (within 3 months of screening) or current use of obeticholic acid/Ocaliva, systemic corticosteroids, methotrexate, tamoxifen, amiodarone, or long-term use of tetracyclines.
46. Warfarin, heparin, factor Xa inhibitors (dabigatran betrixaban edoxaban, apixaban, and rivaroxaban).
47. Concomitant medications that prolong the QT/QTc interval and are known to be associated with increased risk of Torsade des pointes as identified in the https://crediblemeds.org/ website list category of '**Known Risk'** (Appendix B). For subjects on medications that are listed in Appendix B, approval must be obtained from the Sponsor and Medical Monitor.

7.4 Withdrawal of Subjects

A subject may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons.

Reasons for withdrawal (subjects who refuse to return for any remaining study visits) or discontinuation (subjects who prematurely stop the treatment) at any time during the study may include, but are not limited, to the following:

1. Withdrawal of subject consent.
2. Investigator determines that withdrawal from the study is in the best interest of the subject.
3. Major protocol violation (i.e., circumstances where confounding conditions make it impossible to derive sound scientific or medical conclusions from the primary endpoint data generated on a subject).
4. Any condition, injury, or disease that becomes apparent during the study and necessitates the termination of the subject from the study.
5. The development of an elevated temperature is not a cause for withdrawal of HU6 alone. Should a subject develop a temperature > 100°F, they should be instructed to repeat the measurement approximately 15 minutes later. If the temperature continues to be elevated, the subject should be instructed to contact the investigational site to be evaluated by the investigator. They should not take HU6 nor any anti-pyretic (acetaminophen, aspirin, nonsteroidal anti-inflammatory drugs) prior to evaluation by the investigator. The investigator should assess the subject medically to ascertain the underlying cause of the elevated temperature. Appropriate laboratory tests, including those for COVID-19 or influenza, may be warranted to provide a diagnosis.
    a) Should the investigator be unable to identify the cause for the elevated temperature, but the subject's body temperature has returned to normal, dosing with HU6 may be restarted based on the medical judgment of the investigator and in discussions with the medical monitor. Appropriate follow-up should take place once dosing re-starts.

b) Subjects with persistent temperature elevations after HU6 has been stopped for 3 days may be discontinued from the study to allow appropriate medical care, as medically indicated. Restarting dosing with HU6 in subjects who have not taken the drug for greater than 4 days should be discussed with the medical monitor, as their participation in the study will be discontinued.

6. Increased liver function tests will be evaluated in accordance with the Consensus guidelines for best practices for the detection, assessment, and management of suspected acute drug-induced liver injury (DILI) during clinical trial in patients with nonalcoholic steatohepatitis which are applicable to the subjects being enrolled in this trial (Regev et al., 2018). Please see Appendix C for a table from that Consensus that shows the algorithm for monitoring and managing of hepatocellular DILI in phase 2-3 NASH clinical trials in patients with normal or elevated baseline ALT, as well as the listing of the Consensus and Recommendations.
7. Administrative reason (e.g., termination of the clinical study by a Regulatory Agency or the Sponsor).
8. Lost to follow-up.
9. Pregnancy.

7.4.1 Follow-up Procedures for Subjects Who Withdraw Prematurely

Subjects not completing the entire study should be fully evaluated when possible. All withdrawn subjects will be asked to report to the clinic for the Early Termination Visit within 2 weeks after their last dose of the study drug.

Subjects with ongoing AEs or AEs believed to be at least possibly related to study medication will continue to be followed until resolution or for 30 days as warranted by the nature of the AE.

The Investigator must make every attempt to follow-up on subjects who have withdrawn for any reason. When a subject is "lost to follow-up" (i.e., fails to return for study visits), a reasonable effort (3 documented phone calls or 2 phone calls and 2 text messages, on separate occasions, and a follow-up letter sent by registered mail) should be made to contact him/her to determine a reason for the failure to return. If the subject cannot be reached, they should be identified as "lost to follow-up" in the eCRF.

7.4.2 Procedures for Replacing Subjects Who Withdraw Prematurely

A randomized subject who withdraws prior to first dose of study drug may be replaced after consultation with the Sponsor. This subject will be given the same treatment assignment (by the unblinded statistician) as the withdrawn subject. A randomized subject who withdraws from the study after initiation of study drug dosing will not be replaced.

8 TREATMENT PLAN AND METHODS

Study procedures should be completed as designated in the Schedule of Assessments (Section 8.1). However, if a participant is unable to attend a visit within a specified window, the Investigator (or qualified designee) should discuss appropriate scheduling with the Sponsor's Medical Monitor.

Blood samples for HU6, DNP, and M1 PK are required for analysis of parameters and these samples should be collected as close to the protocol-defined times for sample collection as possible.

Whenever 12-lead ECGs, routine vital sign measurements, and blood draws are scheduled for the same time, it is preferable (but not required) that these assessments occur in the following order: 12-lead ECGs, vital signs, and blood draws. The timing of the assessments should allow the blood draws to occur at the approximate nominal time. The actual date and time should be recorded for all procedures and the Investigator should make every effort to perform procedures at the nominal dates and times. However, unscheduled procedures required for the evaluation of safety concerns take precedence over all scheduled routine procedures.

8.1 Schedule of Assessments

	Screening[a]	Treatment Visits									Follow-up	Early Termination Visit	
	Visit 1	Visit 2		Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9[b]	Visit 10		
Activity/Day	Day -45 to -3	Day 1: Predose	Day 1: Postdose	Day 2	Day 7	Day 14	Day 28	Day 42	Day 49	Day 61[b]	Day 73	< 28 days of dosing	≥ 28 days of dosing
Visit Window					±1	±1	±4	±4	±2	varies[b]	±2		
Informed Consent	X												
Inclusion / Exclusion Criteria	X	X											
Medical History	X	X											
Alcohol use assessment	X												
Blood PEth Test	X						X						
TSH and T4	X												
Serum triglycerides	X												
HbA_{1c}	X									X		X	X
Fibroscan	X									X		X	X
Demographics	X												
eGFR (CKD-EPI)	X												
Coagulation (INR)	X												
Serology: HIV, HBsAg, HCV Ab	X												
Urine Drug	X	X					X			X			
Urine Pregnancy test (female only)	X	X			X	X	X	X		X	X	X	X
Laboratory U/A (dipstick)	X	X					X			X	X	X	X
Hematology Panel (CBC)	X	X					X			X	X	X	X
Serum chemistry (includes LFTs)	X	X					X			X	X	X	X

	Screening[a]	Treatment Visits									Follow-up	Early Termination Visit	
	Visit 1	Visit 2		Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9[b]	Visit 10		
Activity/Day	Day-45 to -3	Day 1: Predose	Day 1: Postdose	Day 2	Day 7	Day 14	Day 28	Day 42	Day 49	Day 61[b]	Day 73	< 28 days of dosing	≥ 28 days of dosing
Visit Window					±1	±1	±4	±4	±2	varies[b]	±2		
Ophthalmologic exam	X									X			X
Vital signs[c] and weight (InBody scale)	X	X	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG	X	X			X	X	X	X		X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X
MRI/MRI-PDFF	X[d]									X			X
Complete physical (includes neuro exam: muscle strength)	X									X			X
Targeted physical (includes neuro exam: muscle strength)		X				X	X	X			X	X	
Glycated Albumin		X				X	X	X		X		X	X
hs-CRP		X					X			X			X
Lipid Panel (HDL, LDL, VLDL, FFA, cholesterol, triglycerides)		X					X			X			X
ApoB and Lp(a)		X					X			X			X
HOMA-IR: glucose, insulin, and C-peptide (3 draws 5 minutes apart)		X					X			X			X
OWLiver (Metabolomics)		X					X			X			X
OWL (Lipidomics)		X					X			X			X
Proteomic Profile (SomaScan)		X					X			X			X

	Screening[a]	Treatment Visits									Follow-up	Early Termination Visit	
	Visit 1	Visit 2		Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9[b]	Visit 10		
Activity/Day	Day-45 to -3	Day 1: Predose	Day 1: Postdose	Day 2	Day 7	Day 14	Day 28	Day 42	Day 49	Day 61[b]	Day 73	< 28 days of dosing	≥ 28 days of dosing
Visit Window					±1	±1	±4	±4	±2	varies[b]	±2		
Enhanced Liver Fibrosis Assay (ELF)		X								X			X
Exploratory biomarker (Serum sample)		X					X			X			X
Exploratory biomarker (Plasma sample)		X					X			X			X
Plasma sampling for PK[e]		X	X	X	X	X	X	X		X	X	X	X
Dispense study drug				X	X	X	X	X	X				
Administer study drug		X		X	X	X	X	X	X	X			
Dispense Daily Log (dosing, temperature, diet)				X	X	X	X	X	X				
Collect Daily Log (dosing, temperature, diet)					X	X	X	X	X	X		X	X

a. Subjects will be screened over a 45 day period to determine their eligibility based on specific history, physical, laboratory and imaging evaluations as per the Schedule of Assessments. Due to scheduling of the procedures, multiple visits will likely be necessary to complete the screening process. However, if all screening assessments and procedures can be completed within 30 days of first dose, then a single screening visit is permissible.
b. Visit 9 may be split into a series of visits to complete all assessments. Please see Section 8.2.9. Administering study drug: if the visit occurs on Day 61. For those subjects whose visit occurs on Day 62, the last dose should be taken on Day 61, with no drug administered on Day 62.
c. Vital signs (body temperature, systolic and diastolic blood pressure, heart rate, and respiration rate) conducted twice (predose and prior to discharge) on Days 1, 14, and 28. Only predose vital sign assessments on other Treatment Visit Days.
d. By exception, MRI must be 30 days prior to Day 1 dosing (see Section 8.2.1)
e. PK Sampling: On Day 1, 14, and 28: predose, and approximately 2, 4, and 6 hours. On Days 2, 7, 42, and 61: predose sample. One sample during Visit 10 or Early Termination.

8.2 Study Specific Procedures

Below is a list of procedures per visit. The description for each assessment can be found in the specific sections.

8.2.1 Screening

Subjects will be screened over a 45 day period to determine their eligibility based on specific history, physical, laboratory and imaging evaluations as per the Schedule of Assessments. Due to scheduling of the procedures, multiple visits will likely be necessary to complete the screening process. The screening assessments are outlined in Section 8.2.1.1 below. However, a single screening visit or amended screening visit schedule may be possible as follows:

- If all screening assessments and procedures can be completed within 30 days of first dose, then a single screening visit is permissible.
- The baseline MRI must be conducted within 30 days of Day 1 dosing.
- The Investigator may repeat vital signs, ECGs and laboratory tests once to verify results as based on clinical judgment at the time of the subject screening evaluation.

8.2.1.1 Visit 1 / Screening – (-45 days to -3 days)

Screening assessments will occur within -45 to -3 days prior to Visit 2 / Day 1.

The following procedures will be performed:

- Obtain signed Informed Consent Form.
- Measure and record vital signs and InBody Scale for weight (Section 11.3).
- Evaluate subject eligibility against study inclusion/exclusion criteria.
- Record demographics and ethnicity.
- Obtain medical history including medications.
- Alcohol use assessment (history; current consumption; AUDIT Score) (Appendix A).
- Perform single 12-lead ECG (Section 11.6).
- Draw and process blood samples for laboratory tests (Section 11.5):
    - Comprehensive metabolic panel (includes liver function tests [LFTs] as well as routine chemistry panel, glucose, CPK, magnesium)
    - Hematology (CBC)
    - Serology (HIV, HepB, HCV)
    - Coagulation (INR)
    - eGFR (CKD-EPI)
- Collect and process urine sample for laboratory tests (Section 11.5):

- Routine UA (dipstick)
- Pregnancy test (female subjects)
- Drug screen

- Perform complete physical examination including a neurological examination (Section 11.2).
- Draw blood for laboratory tests (Section 11.5):
  - PEth Test
  - Serum triglycerides concentration
  - HbA_{1c}
  - TSH, T4
- Record concomitant medications.
- Record any AEs.

If eligible, continue with the following screening assessments:

- Perform Fibroscan prior to MRI-PDFF to assess eligibility. (Section 10.3).
- Perform Ophthalmologic examination (Section 11.3).
- Perform MRI/MRI-PDFF (Section 10.1) with 30 days of Day 1 dosing

If eligible, schedule subject to return to for the Visit 2 / Day 1 to initiate HU6 dosing.

8.2.2 Baseline Visit 2 / Day 1

Subjects will have predose assessments at Visit 2 / Day 1 prior to administering the first dose of study drug. Subjects should present to this study visit in the fasted state (minimum 8-hour overnight fast).

The following **predose procedures/assessments** will be conducted on Day 1:

- Confirm eligibility against study inclusion/exclusion criteria.
- Medical history to determine if any changes have occurred since the Screening visit was performed.
- Draw and process blood samples for laboratory tests (Section 11.5):
  - Hematology (CBC)
  - Comprehensive metabolic panel (includes LFT as well as routine chemistry panel; glucose, CPK, magnesium)
  - Glycated albumin
  - hs-CRP
  - Low-density lipoprotein (LDL), very low-density lipoproteins (VLDL), high-density lipoprotein (HDL), FFA, total cholesterol, and triglycerides.
  - ApoB and Lp(a)
  - HOMA-IR - includes glucose, insulin, and C-peptide

- Collect and process urine sample for laboratory tests (Section 11.5):
    - Routine UA (dipstick)
    - Pregnancy test (female subjects)
    - Drug screen
- Draw and process blood samples for PD assessments:
    - OWL metabolomics and OWL lipidomics (Section 10.4)
    - SomaScan proteomics (Section 10.5)
    - ELF assay (Section 10.6)
    - Exploratory biomarkers (Section 10.7)
- Draw and process blood sample for PK (Section 9.1).
- Perform targeted physical examination including a neurological examination (Section 11.2).
- Perform 12-lead ECG (Section 11.6).
- Measure and record vital signs including the InBody scale (Section 11.3).
- Record AEs or changes in medications since the Screening visit.

Administer study drug (Section 6.2.1) after completion of all predose assessments. Conduct the following **postdose procedures/assessments:**

- Draw and process blood samples for PK (Section 9.1) at approximately 2, 4, and 6 hours postdose.
- Measure and record vital signs (Section 11.3) prior to discharge.
- Record AEs and concomitant medications.

8.2.3 Visit 3 / Day 2

Subjects should present to this study visit in the fasted state (minimum 8-hour overnight fast).

The following **predose procedures/assessments** will be conducted on Day 2:

- Record AEs and concomitant medications.
- Measure and record vital signs including the InBody scale (Section 11.3).
- Collect and process blood sample for PK (Section 9.1).

Administer study drug (Section 6.2.1) after completion of all predose assessments. Conduct the following **postdose procedures/assessments:**

- Record AEs and concomitant medications.
- Dispense study drug.
- Dispense Daily Dosing Log.

8.2.4 Visit 4 / Day 7 (±1 day)

Subjects should present to this study visit in the fasted state (minimum 8-hour overnight fast).

The following **predose procedures/assessments** will be conducted:

- Collect and Review Daily Dosing Log.
- Measure and record vital signs including the InBody scale (Section 11.3).
- Perform 12-lead ECG (Section 11.6).
- Collect urine sample for pregnancy test (female subjects [Section 11.5]).
- Draw and process blood sample for PK (Section 9.1).
- Record AEs and concomitant medications.

After completing predose assessments:

- Administer study drug (Section 6.2.1) after completion of all predose assessments.
- Dispense study drug.
- Dispense Daily Dosing Log.

8.2.5 Visit 5 / Day 14 (±1 day)

Subjects should present to this study visit in the fasted state (minimum 8-hour overnight fast).

The following **predose procedures/assessments** will be conducted:

- Collect and Review Daily Dosing Log.
- Measure and record vital signs including the InBody scale (Section 11.3).
- Perform 12-lead ECG (Section 11.6).
- Record AEs and concomitant medications.
- Perform targeted physical examination including a neurological examination (Section 11.2).
- Collect urine sample for pregnancy test (female subjects [Section 11.5]).
- Draw and process blood sample for glycated albumin (Section 11.5).
- Draw and process blood sample for PK (Section 9.1).

Administer study drug (Section 6.2.1) after completion of all predose assessments. Conduct the following **postdose procedures/assessments:**

- Draw and process blood samples for PK (Section 9.1) at approximately 2, 4, and 6 hours postdose.
- Record AEs and concomitant medications.
- Measure and record vital signs (Section 11.3) prior to discharge.
- Dispense study drug.
- Dispense Daily Dosing Log.

8.2.6 Visit 6 / Day 28 (±4 days)

Subjects should present to this study visit in the fasted state (minimum 8-hour overnight fast).

The following **predose procedures/assessments** will be conducted:

- Collect and Review Daily Dosing Log.
- Draw and process blood samples for laboratory tests (Section 11.5):
    - Hematology (CBC)
    - Comprehensive metabolic panel (includes LFT as well as routine chemistry panel; glucose, CPK, magnesium)
    - Glycated albumin
    - hs-CRP
    - LDL, VLDL, HDL, FFA, total cholesterol, and triglycerides
    - ApoB and Lp(a)
    - HOMA-IR - includes glucose, insulin, and C-peptide
    - PEth test
- Collect and process urine sample for laboratory tests (Section 11.5):
    - Routine UA (dipstick)
    - Pregnancy test (female subjects)
    - Drug screen
- Draw and process blood samples for PD assessments:
    - OWL metabolomics and OWL lipidomics (Section 10.4)
    - SomaScan proteomics (Section 10.5)
    - Exploratory biomarkers (Section 10.7)
- Draw and process blood sample for PK (Section 9.1).
- Perform targeted physical examination including a neurological examination (Section 11.2).
- Measure and record vital signs including the InBody scale (Section 11.3).
- Perform 12-lead ECG (Section 11.6).
- Record AEs and concomitant medications.

Administer study drug (Section 6.2.1) after completion of all predose assessments. Conduct the following **postdose procedures/assessments:**

- Draw and process blood samples for PK (Section 9.1) at approximately 2, 4, and 6 hours postdose.
- Measure and record vital signs (Section 11.3) prior to discharge.
- Record AEs and concomitant medications.

- Dispense study drug.
- Dispense Daily Dosing Log.

8.2.7 Visit 7 / Day 42 (±4 days)

Subjects should present to this study visit in the fasted state (minimum 8-hour overnight fast).

The following **predose procedures/assessments** will be conducted:

- Collect and Review Daily Dosing Log.
- Measure and record vital signs including the InBody scale (Section 11.3).
- Perform 12-lead ECG (Section 11.6).
- Record AEs and concomitant medications.
- Perform targeted physical examination including a neurological examination (Section 11.2).
- Collect urine sample for pregnancy test (female subjects [Section 11.5]).
- Draw and process blood sample for glycated albumin (Section 11.5).
- Draw and process blood sample for PK (Section 9.1).

After completing predose assessments:

- Administer study drug (Section 6.2.1).
- Dispense study drug.
- Dispense Daily Dosing Log.

8.2.8 Visit 8 /Day 49 (±2 days)

The following procedures will be conducted:

- Collect and Review Daily Dosing Log.
- Measure and record vital signs including the InBody scale (Section 11.3).
- Record AEs and concomitant medications.
- Administer study drug (Section 6.2.1).
- Dispense study drug.
- Dispense Daily Dosing Log.

8.2.9 Visit 9 / Day 61

Visit 9 may be split into multiple visits to allow for conduct of all procedures and assessments. The allowable visit windows along with specific assessments/procedures to be conducted within these visit windows is detailed below.

8.2.9.1 Visit 9a / Day 61 (±1 day)

On Study Day 61 (±1 day), the following assessments will be conducted:

Subjects should present to this study visit in the fasted state (minimum 8-hour overnight fast).

The following **predose procedures/assessments** will be conducted:

- Collect and Review Daily Dosing Log.
- Draw and process blood samples for laboratory tests (Section 11.5):
    - Hematology (CBC)
    - Comprehensive metabolic panel (includes LFT as well as routine chemistry panel; glucose, CPK, magnesium)
    - HbA_{1c}
    - Glycated albumin
    - hs-CRP
    - LDL, VLDL, HDL, FFA, total cholesterol, and triglycerides
    - ApoB and Lp(a)
    - HOMA-IR - includes glucose, insulin, and C-peptide
- Collect and process urine sample for laboratory tests (Section 11.5):
    - Routine UA (dipstick)
    - Pregnancy test (female subjects)
    - Drug screen
- Draw and process blood samples for PD assessments:
    - OWL metabolomics and OWL lipidomics (Section 10.4)
    - SomaScan proteomics (Section 10.5)
    - ELF assay (Section 10.6)
    - Exploratory biomarkers (Section 10.7)
- Draw and process blood sample for PK (Section 9.1).
- Perform complete physical examination including a neurological examination (Section 11.2).
- Measure and record vital signs including the InBody scale (Section 11.3).
- Perform 12-lead ECG (Section 11.6).
- Record AEs and concomitant medications.
- Administer study drug (Section 6.2.1) if the visit occurs on Day 61. For those subjects whose visit occurs on Day 62, the last dose should be taken on Day 61, with no drug administered on Day 62.

8.2.9.2 Visit 9b / Day 61 (-4 days to + 1 day)

- Perform MRI/MRI-PDFF (Section 10.1).

8.2.9.3 Visit 9c / Day 61 (-5 days to + 2 days)

- Perform Fibroscan (Section 10.3).

8.2.9.4 Visit 9d / Day 61 (-5 days to + 7 days)

- Perform Ophthalmologic examination (Section 11.3).

8.2.10 Visit 10/ Day 73 (±2 days)

The following procedures will be conducted during the follow-up visit:

- Collect and process urine sample for laboratory tests (Section 11.5):
    - Routine UA (dipstick)
    - Pregnancy test (female subjects)
- Draw and process blood samples for laboratory tests (Section 11.5):
    - Hematology (CBC)
    - Comprehensive metabolic panel (includes LFT as well as routine chemistry panel; glucose, CPK, magnesium)
- Draw and process blood sample for PK (Section 9.1).
- Perform targeted physical examination including a neurological examination (Section 11.2).
- Measure and record vital signs including the InBody scale (Section 11.3).
- Perform 12-lead ECG (Section 11.6).
- Record AEs and concomitant medications.

8.2.11 Early Termination Visit

The early termination visit assessments is based on whether the subject completed < 28 days or ≥ 28 days of treatment. Recognizing that subjects may refuse to complete all the assessments for early termination, the procedures have been arranged in the order of prioritization.

For < 28 days of dosing, the following early termination visit procedures will be conducted:

Subjects should present to this study visit in the fasted state (minimum 8-hour overnight fast).

The following procedures/assessments will be conducted:

- Collect and Review Daily Dosing Log.
- Draw and process blood samples for laboratory tests (Section 11.5):
    - Hematology (CBC)
    - Comprehensive metabolic panel (includes LFT as well as routine chemistry panel; glucose, CPK, magnesium)
    - HbA_{1c}
    - Glycated albumin

- Collect and process urine sample for laboratory tests (Section 11.5):
    - Routine UA (dipstick)
    - Pregnancy test (female subjects)
- Draw and process blood sample for PK (Section 9.1).
- Perform targeted physical examination including a neurological examination (Section 11.2).
- Measure and record vital signs including the InBody scale (Section 11.3).
- Perform 12-lead ECG (Section 11.6).
- Record AEs and concomitant medications.
- Perform Ophthalmologic examination (Section 11.3) (only if subject has been dosed for ≥14 days).
- Perform Fibroscan (Section 10.3).

For ≥ 28 days of dosing, the following early termination visit procedures will be conducted:

Subjects should present to this study visit in the fasted state (minimum 8-hour overnight fast).

The following procedures/assessments will be conducted:

- Collect and Review Daily Dosing Log.
- Perform complete physical examination including a neurological examination (Section 11.2).
- Draw and process blood samples for laboratory tests (Section 11.5):
    - Hematology (CBC)
    - Comprehensive metabolic panel (includes LFT as well as routine chemistry panel; glucose, CPK, magnesium)
    - HbA_{1c}
    - Glycated albumin
    - hs-CRP
    - LDL, VLDL, HDL, FFA, total cholesterol, and triglycerides
    - ApoB and Lp(a)
    - HOMA-IR - includes glucose, insulin, and C-peptide
- Collect and process urine sample for laboratory tests (Section 11.5):
    - Routine UA (dipstick)
    - Pregnancy test (female subjects)
- Draw and process blood samples for PD assessments:
    - OWL metabolomics and OWL lipidomics (Section 10.4)
    - SomaScan proteomics (Section 10.5)
    - ELF assay (Section 10.6)

- Exploratory biomarkers (Section 10.7)

- Draw and process blood sample for PK (Section 9.1).
- Measure and record vital signs including the InBody scale (Section 11.3).
- Perform 12-lead ECG (Section 11.6).
- Record AEs and concomitant medications.
- Perform Ophthalmologic examination (Section 11.3).
- Perform MRI/MRI-PDFF (Section 10.1).
- Perform Fibroscan (Section 10.3).

8.3 Concomitant Medications and Other Restrictions

8.3.1 Concomitant Medications

Subjects must be on stable doses of medications for underlying obesity-related conditions for at least 2 months prior to screening.

Subjects with diabetes may be treated with metformin, DPP-4 inhibitors, or sulfonylureas, but must be on stable doses for at least 2 months prior to screening.

Any concomitant medication use must have a strong clinical indication (i.e. treatment of AEs).

The following are expressly **prohibited** during the study:

a) Any herbal supplement, over the counter drug, mail order or prescription drug for weight loss.

b) Products with CBD.

c) Prescription or over the counter stimulants including: dextroamphetamine/Dexedrine, dextroamphetamine/amphetamine combination product/Adderall, or methylphenidate (Ritalin®, Concerta®).

d) Thiazolidinediones (TZD): pioglitazone/Actos, rosiglitazone/Avandia.

e) Glucagon-like peptide 1 (GLP1) agonists: exenatide/Byetta/Bydureon, lixisenatide/Adlyxin, liraglutide/Victoza, dulaglutide/Trulicity, semaglutide/Ozempic.

f) Sodium-glucose cotransporter-2 (SGLT2) inhibitors: canagliflozin/Invokana, dapagliflozinfarxiga, empagliflozin/Jardiance, ertugliflozin/Steglatro.

g) Vitamin E: use of ursodiol or high dose vitamin E >400 IU/day for at least one month within in the last 6 months or started high dose vitamin E within last 3 months of screening.

h) Obeticholic acid/Ocaliva, systemic corticosteroids, methotrexate, tamoxifen, amiodarone, or long-term use of tetracyclines.

i) Warfarin, heparin, factor Xa inhibitors (dabigatran betrixaban edoxaban, apixaban, and rivaroxaban).

j) Concomitant medications that prolong the QT/QTc interval and are known to be associated with increased risk of Torsade des pointes as identified in the https://crediblemeds.org/ website list category of '**Known Risk'** (Appendix B).

8.3.2 Contraception and Pregnancy Avoidance Measures

Reproductive toxicology studies have not been yet conducted and the risks of **HU6** on the fetus are unknown.

Male subjects are required to use one of the following acceptable forms of contraceptives during the study until 30 days after the last dose of study drug if they engage in sexual intercourse with a woman of childbearing potential:

- Condoms
- Sexual abstinence
- Vasectomy

For female subjects, non-childbearing potential requires being either surgically sterile (e.g., hysterectomy, bilateral tubal ligation, bilateral oophorectomy) or post-menopausal ≥12 months of spontaneous and continuous amenorrhea with a follicle stimulating hormone (FSH) level > 40 IU/L in a female).

Women of childbearing potential are required to use one of the following acceptable forms of contraceptives during the study until final follow-up visit:

- Double-barrier methods of contraception; condoms with the use of caps (with spermicide) or with the use of intrauterine device (IUD) are acceptable.
- Hormonal contraceptives (oral, depots, patches, etc.) with a barrier method of contraception.
- Abstinence from sexual intercourse.
- A male partner who is sterile prior to the female subject's entry into the study and is the sole sexual partner for that female subject.
- Is with a same-sex partner and does not participate in bisexual activities where there is any risk of pregnancy.

Male subjects must not donate sperm from day 1 of dosing and for at least 30 days after the last dose of study drug.

Female subjects of childbearing potential must not donate ova during the study and for at least 30 days after the last dose of study drug.

8.3.3 Lifestyle and Dietary Restrictions

Subjects will be instructed to maintain their same diet and activity/exercise level throughout the study as they had prior to participation in the study.

- Subjects should continue their typical dietary habits during study conduct. There is no need for dietary changes. Subjects should not start a weight reduction program while participating in the study.

- Subjects should continue their typical exercise routine during study conduct. However, strenuous exercise should be avoided for 48 hours prior to each blood collection for clinical laboratory tests. Strenuous exercise includes strength (weightlifting, barre, etc.) and speed-strength (boot camp, cross fit, etc.) activities. Exercise such as walking or a slow jog is permitted as long as it is within the subject's typical regimen.

Subjects must not smoke or vape more than 10 cigarettes per week and cannot smoke or vape starting 1 day before each study visit. Nicotine patches are allowed.

Subjects should avoid ingesting alcohol for 48 hours prior to each blood collection for clinical laboratory tests. At other times during the study, alcohol intake should not exceed 14 drinks per week for men and 7 drinks per week for women [1 drink = 4 ounces (120 mL) of wine or 12 ounces (360 mL) of beer or 1 ounce (30 mL) of hard liquor].

9 PHARMACOKINETIC ASSESSMENTS

9.1 Pharmacokinetic Sample Collection

Blood samples will be collected, and plasma samples prepared at the following times:

- On Day 1, 14, and 28: predose (trough) and approximately 2, 4, and 6 hours postdose.
- On Days 2, 7, 42, and 61: predose (trough) sample.
- One sample during Visit 10 or Early Termination.

9.2 Handling, Shipping, Storage and Analysis of Blood Samples

Please refer to the Laboratory Procedures Manual for more information about sample collection, processing, storage, and shipment.

9.3 Pharmacokinetic Parameters

The following PK parameters will be determined from concentration-time data:

- Population PK (PPK) analysis of HU6 parent and metabolites (DNP and M1). The following PK parameters will be calculated: C_{max}, T_{max}, $t_{½}$, T_{lag}, AUC_{0-t}, $AUC_{0-\infty}$, CL/F, Vd/F, λz. Other PK parameters may be calculated, as data allow and appropriate.
- As data permit, noncompartmental analysis of HU6 parent and metabolites (DNP and M1). The following PK parameters will be calculated: C_{max}, AUC, and accumulation.

10 PHARMACODYNAMIC ASSESSMENTS

An overview of PD assessments is provided below. Refer to Section 8 for PD blood collection time points. Please refer to the Study Procedure Manual for more information about sample collection, processing, storage, and shipment.

10.1 Magnetic Resonance Imaging-Proton Density Fat Fraction (MRI-PDFF)

Magnetic Resonance Imaging Proton Density Fat Fraction (MRI-PDFF) is a non-invasive, quantitative biomarker to assess liver fat content (steatosis). The percentage of fat in the liver, or proton density fat fraction (PDFF), is being measured using MR: MRI-PDFF at baseline and end of treatment. Liver volume will be assessed from an axial T1 weighted or dual echo gradient echo images covering the entire liver. This advanced MRI technique measures the fraction of mobile protons in the liver attributable to liver fat (the PDFF), which is a direct measure of liver fat content and is a fundamental tissue property. Subjects should be fasting for 4 hours prior to the MRI-PDFF being performed, however, if they are not, the scan can still be performed. Should the MRI show incidental findings unrelated to the study evaluation, subjects will be notified of the findings and a written report provided and a recommendation for the subject to follow up with their primary physician.

10.2 Abdominal MRI

An MRI image will be taken of the abdominal region to assess total fat. The two primary measurements from this scan will estimate the total visceral adipose tissue (VAT), the type of fat stored within the body cavity, and the subcutaneous adipose tissue (SAT), the type of fat visible right underneath the skin.

10.3 Fibroscan®

The Fibroscan is a non-invasive medical device which estimates liver fat content (steatosis) and liver stiffness (fibrosis). The assay works by measuring shear wave velocity. A 50-MHz wave is passed into the liver from a small transducer on the end of an ultrasound probe. The probe also has a transducer on the end that measure the velocity of the shear wave (in meters per second) as this wave passes through the liver. The shear wave velocity is converted into liver stiffness, which is expressed in kilopascals (VCTE score). A second measurement is also taken that estimates hepatic steatosis through measuring the ultrasonic attenuation of the echo wave, termed the controlled attenuation parameter (CAP). Subjects will need to be fasting for 4 hours prior to the Fibroscan® being conducted.

10.4 One Way Liver Assays (OWL)

10.4.1 OWL – Metabolomics

This metabolomics assay extracts metabolites from volunteer plasma and serum to take a snapshot of cellular function. Liquid chromatography-mass spectrometry (LC-MS) metabolomics is used to identify serum biomarkers that differentiate normal liver and NAFLD and between NASH and NAFLD. The metabolomics profile also provides insight into cellular function and inflammation through the examination of various cellular metabolites that provide insight into key molecular pathways.

10.4.2 OWL – Lipidomics

Lipidomics is non-invasive blood assay that analyzes and identifies lipids in plasma and serum. These lipids will be separated and characterized via mass spectrometry and the analysis will include fatty acids, fatty acid derivatives, glycerolipids, glycerophospholipids, sphingolipids, and sterols.

10.5 Slow Off-rate Modified Aptamer (SomaScan)

SomaScan is a non-invasive blood assay. Blood plasma and serum samples will be assayed using oligonucleotide aptamers whose three-dimensional conformational shape binds specifically to a protein target of interest. Over seven thousand proteins will be assayed and quantified, enabling a proteomic snapshot of the body. Different mathematical models have been applied to large clinical data to establish algorithms with predictive value in cardiovascular health, metabolic rate, lean body mass, liver inflammation, cardiorespiratory fitness, and glucose tolerance.

10.6 Enhanced Liver Fibrosis (ELF)

The ELF assay is a non-invasive blood test that measures three markers of liver inflammation and fibrosis: hyaluronic acid, procollagen III amino-terminal peptide (PIIINP), and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1). The values of these three markers, when used in conjunction with accompanying clinical data, are highly predictive of the inflammatory and fibrotic state of the liver, as evidenced by correlating the data with histology in larger clinical trials.

10.7 Exploratory Biomarkers

Blood samples will be obtained and separated into plasma and serum components from which separate 400 µL aliquots will be drawn, labelled, and stored for future analysis of proteins, lipids, or gene expression that could assist in explaining the pharmacological actions of HU6.

11 SAFETY ASSESSMENTS

11.1 Adverse Events

Adverse events will be captured from the start of study-related procedures at Screening (including diagnostic assessments or after signing of ICF) onward during this study. Important medical events and conditions occurring prior to this period are not AEs; they will be captured within the medical chart and in the Medical History section of the Case Report Form.

11.2 Physical Examination

Height should be measured on a wall-mounted stadiometer at Screening.

Refer to Section 8 for physical examination time points.

Complete Physical: At study visits in which a complete physical examination is required, the investigator should perform a thorough examination of all body systems (exception: genitourinary and reproductive should be symptom-directed). Neurological examination should include the assessment of strength.

Targeted Exam: A targeted physical exam that is symptom-based. Based upon change to subject symptoms or visual findings; in addition, each exam should include a limited neurological evaluation of the strength of muscle groups. The commonly accepted method of evaluating muscle strength is the Medical Research Council Manual Muscle Testing scale. This method involves testing key muscles from the upper and lower extremities against the examiner's resistance and grading the patient's strength on a 0 to 5 scale accordingly:

- 0 No muscle activation
- 1 Trace muscle activation, such as a twitch, without achieving full range of motion
- 2 Muscle activation with gravity eliminated, achieving full range of motion
- 3 Muscle activation against gravity, full range of motion
- 4 Muscle activation against some resistance, full range of motion
- 5 Muscle activation against examiner's full resistance, full range of motion

11.3 Ophthalmologic Examination

A full medical eye examination including fundus photographs of the posterior pole of eye, OCT of the maculas of both eyes, and slit lamp evaluations will be performed at screening and at the completion of dosing (approximately Day 61) to characterize the subject's baseline status and to monitor any changes from baseline over the course of treatment. Pupillary dilation will be accomplished with 2.5% neosynephrine and 0.5% tropicamide (Mydriacyl) (unless there is a contraindication deemed by the ophthalmologist), one drop in each eye one time in light color eyes and up to two times, 5 minutes apart in dark eyes. A slit lamp is a biomicroscope with a bright light used during an eye exam and assesses different structures at the front of the eye and inside the eye for determination of the health and detection of eye disease. OCT is a non-invasive imaging technique that uses light waves to take cross-section pictures of the retina. Fundus photography involves photographing the rear of the eye. These procedures are standard tests involved in the medical evaluation of the health of the eye and should be conducted by a limited number of coordinating ophthalmologists to ensure consistency of evaluations.

11.4 Vital Signs

Refer to Section 8 for vital sign assessment time points.

Vital signs include body temperature, systolic and diastolic blood pressure, heart rate, and respiration rate. All blood pressure readings must be done with a blood pressure cuff appropriate to the arm size of the subject. A blood pressure cuff that is too small will result in inaccurately high blood pressure determinations. Blood pressure and heart rate recordings will be made after the study subject has been supine for ≥ 5 minutes.

The InBody scale will be used to capture weight, muscle, and body fat. This must be done predose, in the fasted state, and at approximately the same time of day.

Body temperature will also be monitored daily by the subject at home utilizing a Braun Thermoscan 7 inner ear thermometer which will be provided to them. The thermometer provides a color-coded display that displays the temperature as well as indicating normal, elevated (>99.9º F, yellow display), or fever (>103º F, red display) temperatures. There is an audible feedback system that ensures appropriate usage and alerts the user that the temperature has been acquired. Nine previous reading will be recorded on the thermometer. Temperature readings should be done daily at the time of dosing. Should the subject have symptoms that may indicate a fever, they should take their temperature and verify. For temperature elevations ≥100º F, as indicated by the yellow or red display, the subject should stop taking HU6, avoid antipyretics (acetaminophen, aspirin or non-steroidal anti-inflammatory agents) and call the

Investigational Site. Further guidance will be provided by the medical staff (See Section 7.4 Withdrawal of Subjects).

11.5 Clinical Laboratory Tests

Refer to Section 8 for clinical laboratory blood and urine collection time points.

Refer to the Study Procedure Manual for more information about sample collection, processing, storage, and shipment.

At Screening only:

- Viral serology will include testing for the presence of hepatitis B antigen, anti-hepatitis C antibody and anti-HIV antibodies.
- TSH and Free T4.
- eGFR = CKD-EPI Creatinine Equation (NKF 2009; https://www.kidney.org/content/ckd-epi-creatinine-equation-2009).

Hematology testing will include erythrocyte mean corpuscular hemoglobin concentration (MCHC), erythrocyte mean corpuscular volume (MCV), hematocrit, hemoglobin, leukocyte count, and absolute counts of lymphocytes, monocytes, neutrophils, basophils, eosinophils and platelets.

Serum chemistry analyses will include glucose, calcium, albumin, total protein, sodium, potassium, bicarbonate, chloride, magnesium, blood urea nitrogen (BUN), creatinine, alkaline phosphatase, phosphate, uric acid, lactate dehydrogenase, ALT, AST, gamma-glutamyl transferase (GGT), bilirubin (total and direct), amylase, and CPK.

- Baseline ALT and AST = while each value will be recorded, the average of screening and Day 1 predose values will be used as the baseline value in the statistical evaluation of these parameters in the exploratory analysis.

Lipid panel will include total cholesterol, HDL, LDL, VLDL, triglycerides, and FFA.

Additional tests at select time points include glycated albumin, hs-CRP, ApoB, Lp(a), and HOMA-IR (includes glucose, insulin, and C-peptide; Wallace 2004), and PEth test.

- For HOMA-IR, 3 blood samples for the 3 analytes (blood glucose, serum insulin, and C-peptide) will be drawn after a minimum of 5 minutes between each sample.
- The PEth test is a serum biomarker that can assess recent alcohol consumption. The value is dependent on both quantity of alcohol and time from consumption. This will be assessed at screening and Day 28. At the discretion of the investigator, it can be obtained at other times if there is concern for excessive alcohol consumption based on history, symptoms or laboratory evaluations (e.g. elevated liver function tests). The report lists several different assays, but this study will rely on the based on PEth 16.0/18.1 (POPEth) assay, which has been validated.

Urinalysis will consist of dipstick evaluations, with a reflex microscopic evaluation if dipstick shows blood or protein is small (1+), moderate (2+) or large (3+). Spot urine protein and albumin to be done if urine > trace protein on 2 collections.

Urine pregnancy tests will be conducted for female subjects.

Laboratory tests may be repeated once at screening. Additional laboratory evaluation may be performed at the discretion of the investigator in the assessment of an adverse event, as medically warranted.

11.6 12-lead Electrocardiogram

Refer to Section 8 for 12-lead ECG assessment time points.

Single 12-Lead ECG measurements will be obtained after the subject has rested in a supine position for at least 10 minutes. External stimuli should be kept to a minimum. Video games, watching of TV, and talking will not be allowed during this time. A digital ECG machine will be utilized for the trial. If an ECG timepoint coincides with any blood samples, the ECGs will performed ± 10 minutes from obtaining the blood sample at the same timepoint. In addition, whenever possible, subjects should not have a meal within 2 hours prior to an ECG being performed.

The ECGs will be measured using an ECG machine that automatically calculates the heart rate and measures PR, RR, QRS, QT, and QTcF (Fridericia correction formula). The same ECG machine should be used for the same subject throughout the study, if at all possible. ECGs should be conducted in adherence with a research unit SOP acceptable to the Sponsor.

11.7 Adverse Events

11.7.1 Definitions

Adverse Event

An Adverse Event (AE) is any untoward medical occurrence in a subject or clinical investigation subject undergoing a study procedure or administration of a study drug. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the study drug, whether or not considered related to the study drug. Medical conditions present at the initial trial visit that do not worsen in severity or frequency during the trial are defined as baseline conditions (medical history) and are not to be considered AEs.

The sources of AEs include but are not limited to:

- The subject's response to questions about his/her health (a standard nonleading question such as "How have you been feeling since your last visit?" asked at each visit).
- Symptoms spontaneously reported by the subject.
- Investigations and examinations with findings that are assessed by the Investigator to be clinically significant.
- Other information related to the subject's health becoming known to the Investigator.

For surgical or diagnostic procedures, the condition/illness leading to such a procedure is considered as the AE rather than the procedure itself.

Laboratory Abnormality

A laboratory abnormality will be considered an AE when it is clinically significant and suggesting a disease or organ toxicity and which is of a severity requiring active management (i.e., changes of dose, discontinuation of drug, more frequent follow-up, medical treatment or a diagnostic investigation).

Pretreatment Adverse Events

A pretreatment AE is any AE occurring during the pretreatment period (between informed consent and initiation of a study drug dosing).

Treatment-emergent Adverse Events

Treatment-emergent adverse events (TEAEs) are all AEs occurring during the treatment period or a pretreatment AE that worsens in intensity during the treatment period.

11.7.2 Collection and Rating of Adverse Events

During the course of the study (i.e., from the signing of the ICF through the Follow-up Visit plus 30 days for any SAE) all AEs, irrespective of the relatedness to the study drug, will be collected and reported on the Adverse Event Report Form. The seriousness criteria should not be confused with the intensity of the event. In case of an SAE, a Serious Adverse Event Report Form must be completed and transmitted to the Sponsor or designee.

Overdoses and medication errors in the presence of clinical consequences should be recorded as AEs. The clinical consequence should be reported as "[enter AE] due to overdose."

11.7.2.1 Onset Date

The onset date is the date when the first sign(s) or symptom(s) were first noted. For example, if the AE is an abnormal laboratory test (such as "platelets low"), the onset date is the date when the sample was taken. If the subject was hospitalized for meningitis, and symptoms such as fever, headache and nausea started the day before the hospitalization, the onset date is the day symptoms presented versus day of hospitalization.

11.7.2.2 Assessment of Intensity

The intensity of each AE will be rated according to the following 3-point scale:

- **Mild:** Awareness of signs or symptoms, but no disruption of usual activity
- **Moderate**: Event sufficient to affect usual activity (disturbing)
- **Severe**: Inability to work or perform usual activities (unacceptable)

The CTCAE system will be used to assess the severity of the SAEs, AEs.

11.7.2.3 Relationship to Study Drug

The causal relationship of the study drug to an AE will be rated according to the following 4-point scale:

- **Unrelated:** Clearly and incontrovertibly due only to extraneous causes, and does not meet criteria listed under unlikely, possible or probable
- **Unlikely:** Does not follow a reasonable temporal sequence from administration; may have been produced by the subject's clinical state or by environmental factors or other therapies administered

- **Possible:** Follows a reasonable temporal sequence from administration; may have been produced by the subject's clinical state or by environmental factors or other therapies administered
- **Probable:** Clear temporal association with improvement on cessation of study drug or reduction in dose. Reappears upon re-challenge or follows a known pattern of response to the study drug

AEs considered 'unrelated or unlikely' will be considered unrelated causality, whereas 'possible or probable' will be considered related causality.

11.7.2.4 Action Taken

The action taken toward the study drug in response to an AE will be listed as one of the following:

- **None:** no change in study drug regimen due to the event
- **Discontinued:** the study drug was permanently stopped due to the event
- **Interrupted:** the study drug was temporarily interrupted due to the event

11.7.2.5 Outcome of Adverse Event

The outcome of an AE will be recorded as one of the following:

- **Recovered:** fully recovered or the condition has returned to the level observed at baseline
- **Recovered with sequelae:** resulted in persistent or significant disability or incapacity; the nature of the sequelae should be specified
- **Ongoing**
- **Death**

11.7.3 Adverse Event Follow-up

Adverse events requiring therapy must be treated with recognized standards of medical care to protect the health and well-being of the subject.

Any subject who has any AE (whether serious or non-serious) or clinically significant (in the Investigator's opinion) abnormal laboratory test values will be evaluated by the Investigator or a monitoring physician, and will be treated and followed up until the symptoms or values return to normal or acceptable levels, as judged by the Investigator and the Sponsor.

Adverse events that are unresolved at end of study or upon early withdrawal will be tracked at least weekly by site staff until resolution, for 30 days, or until the subject is lost to follow-up (defined as failure to respond to three phone messages left on separate days and one certified letter requesting follow-up).

Subjects will be instructed to inform site staff of any AEs occurring during the 30-day period after discharge or early withdrawal.

Any follow-up information available at the time of the subject's end of study will be included in the clinical study report.

Any SAE that is unexpected and related to the study drug occurring after the end of study should be forwarded to the Sponsor. These cases will be handled and submitted as expedited reports but will not be included in the clinical study report.

11.8 Serious and Other Significant Adverse Events

11.8.1 Definition of a Serious Adverse Event

A serious adverse event is any untoward medical occurrence that:

- **Results in death.** Death is not an event per se but rather an outcome. Note that any event resulting in a fatal outcome must be fully documented and reported, including deaths that occur within 30 days after treatment ends and irrespective of the causal relationship to the study drug.
- **Is life-threatening.** Life-threatening refers to an AE in which the subject was at immediate risk of death at the time of the event. It does not refer to an event, which may have caused death, if it was more severe.
- **Requires in-patient hospitalization or prolongation of existing hospitalization.** Hospitalization means that the subject was admitted to hospital or that existing hospitalization was extended as a result of an event. Hospitalization describes a period of at least 24 hours. Over-night stays for observation; stays at the emergency room or treatment on an outpatient basis does not constitute a hospitalization. However, medical judgment must always be exercised and, when in doubt, the case should be considered serious (i.e. if the case fulfills the criterion for a medically important event). Hospitalization for administrative or social purposes does not constitute an SAE. Hospital admissions and/or surgical operations planned before study inclusion are not considered AEs if the illness or disease existed before the subject were enrolled in the study, provided that the condition did not deteriorate during the study.
- **Results in persistent or significant disability/incapacity.** Disability/incapacity means a substantial disruption of a person's ability to conduct normal life functions. If in doubt, the decision should be left to medical judgment by the Investigator.
- **Is a congenital anomaly/birth defect.** Any congenital anomaly or birth defect observed in any offspring of the subject conceived during treatment with the study drug.
- **Is an important medical event.** Important medical events are events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of important medical events include AEs that suggest a significant hazard, contraindication or precaution, occurrence of malignancy or development of drug dependency or drug abuse. Medical and scientific judgment should be exercised in deciding whether events qualify as medically important.

An AE caused by an overdose or medical error is considered serious if a criterion listed in the definitions above is fulfilled.

The following are <u>not</u> considered SAEs:

- A pre-existing condition that is present prior to or at the start of the study that did not worsen

- Hospitalizations for treatment which were elective or preplanned, for a pre-existing condition unrelated to the indication under study that did not worsen
- Admission to a hospital or other institution for general care, not associated with any deterioration in condition.

11.8.2 Serious Adverse Event Reporting by the Investigator to the Sponsor

Any SAE that occurs after a subject has entered the study, whether related to study drug, must be reported to the Sponsor or the Sponsor's agent immediately (within 24 hours) via telephone or facsimile. If initially reported via telephone, this must be followed-up by a facsimile of the written SAE report. The Investigator must report all SAEs occurring from the time the subject signs the ICF until 30 days after last treatment with the study drug.

A completed Serious Adverse Event Report Form with the best possible details must be transmitted to the Sponsor representative by facsimile within 24 hours of knowledge of the SAE according to contact details as specified below:

Sponsor Representative and Contact Information for SAE Reporting:

Roger Morgan, M.D.
Vice President Medical Affairs
MedSurgPI, LLC
3700 Lark Farm Road
Franklinton, NC 27525
[redacted]

11.8.3 Handling of Follow-up Information

Follow-up information may be required, or additional information may be received by the Sponsor (e.g., evolution of the SAE, other signs or symptoms, final diagnosis, outcome, hospital discharge summary, or autopsy report). The same procedures and timelines as for initial reporting, listed above, should be followed for any follow-up information. If necessary, the study site will be visited to collect additional information.

Follow-up information is required on all SAEs until one of the following criteria is satisfied:

- The outcome of the case is known
- The event is resolved, or the medical condition of the subject is stabilized
- No further information is available
- Sponsor assessment has been finalized

11.8.4 Reporting and Follow-up of Pregnancy

When an Investigator becomes aware of the pregnancy of a female subject, the Investigator must withdraw the subject from the study and follow the pregnancy until termination or until the child is 1 month old. The pregnancy will be reported immediately by telephone and by faxing a completed Pregnancy Report to the Sponsor within 24 hours of knowledge of the event. The pregnancy will not be

processed as an SAE; however, the Investigator should notify the Sponsor or the Sponsor's agent of the outcome of the pregnancy by submitting a follow-up Pregnancy Report. Additionally, if the outcome of the pregnancy meets the criteria for immediate classification of an SAE (e.g., spontaneous or therapeutic abortion, stillbirth, neonatal death, or congenital anomaly), the Investigator will report the event by phone and by faxing a completed SAE Report Form to the Sponsor within 24 hours of knowledge of the event.

11.8.5 Expedited Reporting of Serious Adverse Events

11.8.5.1 Responsibilities

The Sponsor is responsible for ensuring the timely reporting of SAEs to Regulatory Authorities and all Investigators who participate in the clinical development program of the study drug. It is the responsibility of the Investigator to provide the Sponsor with the case information such that reporting timeline demands of applicable Regulatory Authorities can be met.

11.8.5.2 Expedited Reporting

All AEs that are serious, unexpected, and considered related to the study drug judged by either Sponsor or the Investigator require expedited reporting. All available information relevant to the evaluation of the SAE will be reported. Serious adverse events will be considered reportable regardless of whether the study drug was used in accordance with the provisions in the protocol.

Adverse events which are serious, but expected, or those which are not associated with the study drug will only be subjected to expedited reporting if they are required to be reported to an authority according to national requirements.

11.8.5.3 Timelines

Fatal or life-threatening serious unexpected related cases require rapid reporting. Regulatory Authorities shall be notified as soon as possible but no later than 7 calendar days after first knowledge by the Sponsor representative, followed by as complete a report as possible within 8 additional calendar days.

Serious unexpected related cases that are not fatal or life-threatening must be submitted as soon as possible, but no later than 15 calendar days after first knowledge by the Sponsor representative that the case meets the minimum criteria for expedited reporting.

It is the responsibility of the Investigator to support Sponsor activities needed to meet the timelines for Regulatory Authority reporting in the event of an SAE.

12 STATISTICAL METHODS

12.1 General Overview of the Statistical Analysis Plan

Descriptive statistical methods will be used to summarize the data from this study, with statistical testing performed for the efficacy endpoints. Unless stated otherwise, the term "descriptive statistics" refers to number of subjects (n), mean, median, standard deviation, minimum, and maximum for continuous data, and frequencies and percentages for categorical data. Statistical testing, if performed, will be 2-sided and will be performed using a significance (alpha) level of 0.05. There will be no adjustment for multiple comparisons for this exploratory study. All available data for enrolled subjects will be listed by subject.

Unless otherwise noted, the data will be sorted first by subject number and then by date within each subject number. All statistical analyses will be conducted with the SAS® System, version 9.4 or higher.

Additional statistical details will be provided in the statistical analysis plan (SAP).

In the event of higher than anticipated early terminations due to COVID-19, or other reasons, Rivus Pharmaceuticals, may extend enrollment in order to maintain the planned statistical power.

12.2 Sample Size Determination

A sample size of 17 subjects per group provides at least 80% power to detect a 30% difference in the mean relative change from baseline in liver fat content at Day 61, when the standard deviation is 30% [PASS 2020: Two sample t-test, alpha=.05]. Assuming a 15% dropout rate, up to 80 subjects should be enrolled to ensure that at least 68 subjects, 17 per group, are evaluable for the primary efficacy endpoint analysis.

12.3 Data Analysis

12.3.1 Analysis Populations

Intent-to-Treat (ITT): includes all randomized subjects.

Full Analysis Set (FAS): includes all randomized subjects who receive at least 1 dose of treatment.

PK Concentration Population: includes all subjects who receive at least one dose of HU6 and have at least one adequately documented plasma sample obtained and analyzed for measurable HU6, DNP and M1 concentrations.

PK Parameter Population: includes all subjects who have an evaluable HU6, DNP and M1 plasma concentration data.

12.3.2 Efficacy Analysis

The primary efficacy endpoint is the reduction in liver fat content measured by the relative change from baseline, as assessed by MRI-PDFF at Day 61. The hypothesis of interest are 2-sided tests comparing each active HU6 treatment groups to the placebo.

$H_{01}: \mu_{placebo} = \mu_{HU6\ 170mg}$ vs $H_{11}: \mu_{placebo} \neq \mu_{HU6\ 170mg}$
$H_{02}: \mu_{placebo} = \mu_{HU6\ 340mg}$ vs $H_{12}: \mu_{placebo} \neq \mu_{HU6\ 340mg}$

$$H_{03}: \mu_{placebo} = \mu_{HU6\ 510mg} \text{ vs } H_{13}: \mu_{placebo} \neq \mu_{HU6\ 510mg}$$

Relative change from baseline in liver fat content, will be analyzed for the FAS using an analysis of variance (ANCOVA) model, including treatment group, HbA_{1c} stratification (normal range versus between 5.7% and 9.0% inclusive) and baseline liver fat from MRI-PDFF as fixed effects. This estimand will include available MRI-PDFF evaluations that occur before discontinuation of treatment. The differences between treatment groups will be based on the least square mean (LSMean) differences between each HU6 group and placebo. A separate comparison of the combined HU6 groups and placebo may be conducted. The corresponding 95% confidence interval (CI) will be presented.

12.3.3 Pharmacodynamic Analysis

Continuous pharmacodynamic endpoints with repeated post-baseline assessments (changes from baseline in body weight, whole body adiposity, AVCTE, CAP, ELF, serum hs-CRP, Lp(A), Apo B, LDL, HDL, total cholesterol, triglycerides, FFA, plasma ceramides, HOMA-IR, fasting blood glucose concentrations, glycated albumin concentrations, and HbA_{1c}) will be analyzed for the FAS using a Mixed Model for Repeated Measures (MMRM). This estimand will include available post-baseline response data that occur before discontinuation of treatment. The baseline score will be included as the covariate, and HbA_{1c} stratification, treatment group, visit, and the interaction between treatment group and visit will be included as fixed factors in the MMRM. The difference between each HU6 level versus placebo at each visit will be estimated based on the LSMeans from the MMRM.

12.3.4 Safety Analysis

The safety analysis will be conducted on the FAS. Should there be any subjects that receive a treatment other the randomized treatment, the treatment as received will be used for safety presentations. AEs will be assessed for toxicity grade using NCI CTCAE (Version 5 or higher) and will be coded for summarization using Medical Dictionary for Regulatory Activities (MedDRA® Version 10.1 or higher). Concomitant medications will be coded using WHO Drug Dictionary (enhanced) Format C, 15 Aug 2005 or more recent updated version.

Treatment-emergent AEs will be summarized by SOC and preferred term, for each dose group (including placebo). Further summaries by seriousness, severity, and relationship to study drug may be conducted.

Laboratory measures will be summarized by treatment group and time-point both as absolute values and as change from baseline, with descriptive statistics summarizing each group and time point. Similar presentation will be used for vital signs and for ECG interval measurements, and changes from pre-treatment baseline. ECG intervals will be calculated from ECG/continuous ECG recordings at each time point.

A separate statistical analysis plan may be developed for exploratory evaluations of HU6 concentrations and QTcF.

12.3.5 Pharmacokinetic Analysis

Plasma concentration-time data of HU6, DNP and M1 will be displayed in tables and/or graphs. Individual plasma concentration-time data will be pooled, and population pharmacokinetics will be performed using a currently acceptable methods as permitted by the data and based on a prospective analysis plan. PK parameters such as Clearance (CL) and volume of distribution (V) will be determined. In addition, the influence of various covariates (e.g., BMI) of the PK parameters will be examined. Individual PK parameter estimates will be used to calculate summary metrics of exposure including AUC and C_{max}.

Full details will be provided in a Pharmacokinetic Analysis Plan.

12.3.6 Pharmacokinetic/Pharmacodynamic Analyses

PK/PD modeling may be conducted to better define exposure-response and dose determination for future studies, as appropriate.

12.4 Missing, Unused and Spurious Data

No imputation will be applied for missing data. Only non-missing values will be used for analyses.

13 STUDY MANAGEMENT

13.1 Protocol Amendment and Protocol Deviation

13.1.1 Protocol Amendment

Administrative amendments to the protocol will be classed as amendment of typographical errors, clarifications of confusing wording, and other minor modifications including but not limited to name, address, and contact information changes that have no impact on the safety of the subject or the science of the study. Administrative amendments will be submitted to the Institutional Review Board (IRB) for information only. The Sponsor will ensure that acknowledgement is received and filed. Otherwise, an amendment will be classed as a substantial amendment and will be submitted to the appropriate Regulatory Authorities and the IRB for approval.

13.1.2 Protocol Deviations

No deviations from the protocol are anticipated. Requests for deviations must be made in advance with the Sponsor. Should a non-anticipated protocol deviation occur, the Sponsor must be informed as soon as possible. All deviations and the reasons for the deviation will be documented by the Investigator or designated staff. Reporting of protocol deviations to the IRB and in accordance with applicable Regulatory Authority mandates is an Investigator responsibility.

13.1.3 Protocol Waivers

Protocol waivers will not be granted by the Sponsor in this study.

13.2 Ethics and Regulatory Aspects

13.2.1 Ethical Conduct of the Study and Regulatory Guidelines

To ensure the ethical conduct of this clinical study, each Investigator is expected to conduct the study in accordance with the protocol; the United States IND regulations specified under 21 CFR 11, 50, 54, 56, and 312; the ICH Harmonized Tripartite Guideline for Good Clinical Practice (GCP); and the Guidelines of the Declaration of Helsinki. The Investigator will conduct all aspects of the study in accordance with all national, state and local laws of applicable Regulatory Authorities.

The responsibilities of the Sponsor, the Monitor and the Investigator will be as defined in the ICH GCP consolidated guideline, and applicable regulatory requirements in the country where the study takes place. The Investigator is responsible for adhering to the GCP responsibilities of Investigators, for dispensing the study drug in accordance with the approved protocol or a signed amendment, and for its secure storage and safe handling throughout the study.

13.2.2 Institutional Review Board and Regulatory Approval

The study protocol and any amendments will be reviewed by an Independent Review Board. The IRB will review the written subject information sheet and the Informed Consent Form (ICF), their updates (if any), and any written materials given to the subjects. A listing of the membership of the IRB consulted and the name of the committee chair(s) or IRB registry (accreditation) number will be documented within the Investigator File and Trial Master File of the Sponsor.

The Regulatory permission to perform the study must be obtained in accordance with applicable regulatory requirements. All ethics approvals must be obtained, and regulatory obligations met before a subject is exposed to any study-related procedure, including screening tests for eligibility.

13.2.3 Subject Informed Consent

Subjects will be informed about the study both verbally and in writing. Each subject will be provided with a written subject information sheet that has been approved by the IRB and will be given a reasonable time to consider the study and to ask any questions they have regarding the study. The ICF must be in a language that the subject can understand.

Only the Investigator, a medically qualified Sub-investigator or a suitably qualified and trained authorized person may be involved in the informed consent process.

The Investigator or their suitable designee will obtain a freely given, written consent from each subject after an appropriate explanation of the aims, methods, potential hazards, and any other aspects of the study which are relevant to the decision of the subject to participate. The Investigator will explain that the subject is completely free to refuse to enter the study or to withdraw from it at any time, without any consequences for their further care and without the need to justify.

The ICF must be signed and dated by the subject before exposure to any study-related procedure, including screening tests for eligibility. The subject will receive a copy of the written subject information sheet and the ICF.

Each subject will be informed that a Monitor, a Quality Assurance Auditor mandated by the Sponsor, or a Health Authority Inspector, in accordance with applicable regulatory requirements, may review his or her source records and health data. Data protection will be handled in compliance with national and local regulations.

If new safety information becomes available and results in significant changes in the risk to benefit assessment, the written subject information sheet will be revised or updated where necessary. Under these circumstances, all subjects (including those already being treated) should be informed of the new information, given a copy of the revised form and allowed to reevaluate their consent to continue in the study.

13.3 End of Study and Regulatory Notification

The study can be terminated in part or in whole at the discretion of the FDA, an applicable Regulatory Authority or the Sponsor.

At the end of the study, the IRBs and Regulatory Authorities will be notified by the Sponsor according to applicable Regulatory requirements.

13.4 Data Protection and Confidentiality

The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with applicable regulatory requirement(s).

13.5 Monitoring

The study will be monitored to ensure that the study is conducted and documented properly according to the protocol, GCP, and all applicable regulatory requirements.

On-site visits will be made at appropriate times during the study. Monitors must have direct access to source documentation in order to check the consistency of the data recorded in the CRF.

The Investigator will make available to the Monitor source documents, medical records, and source data necessary to complete CRFs. In addition, the Investigator will work closely with the Monitor and, as needed, provide them appropriate evidence that the conduct of the study is being done in accordance with applicable regulations and GCP guidelines.

13.6 Quality Assurance and Quality Control

The Sponsor or its designee will perform the quality assurance and quality control activities of this study; however, responsibility for the accuracy, completeness, and reliability of the study data presented to the Sponsor lies with the Principal or Qualified Investigator generating the data.

Prior to the study initiation, the Sponsor will explain the protocol, Investigator's Brochure, and CRFs to Investigators. In addition, the Monitor will be available to explain applicable regulations and to answer any questions regarding the conduct of the study.

At its discretion, the Sponsor may conduct audits as part of the implementation of quality assurance to ensure that the study is being conducted in compliance with the protocol, Standard Operating Procedures, GCP, and all applicable regulatory requirements. Audits will be independent of and separate from the routine monitoring and quality control functions.

The study center may also be compelled to an inspection by a Regulatory Authority.

13.7 Source Data

Source data are defined as information in original records and certified copies of original records of clinical findings, observations, data, or other activities in a clinical study necessary for the reconstruction and evaluation of the study.

Source documents are the original data, documents, and records. Examples include hospital records, laboratory reports, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, other radiographic depictions or displays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical study. All source documents must be reviewed by the PI and the sponsor (or designee) for compliance with GCP.

Study-specific data sheets may be used to document source information that would not normally be collected and documented in the routine management of the subject. Data sheets used for source

documentation must be verified and signed by the Investigator or a delegated study site team member and must be stored and archived in the subject's clinic records (preferably) or in the Investigator File.

The Investigator will permit study-related monitoring, audit(s), IRB review(s), and regulatory inspection(s), with a direct access to all the required source documents and associated records.

14 DATA AND RECORD KEEPING

14.1 Case Report Forms

All data will be entered in a validated electronic data capture system using single data entry. Standard procedures (including following data review guidelines, manual clinical review based on subject profiles, computerized validation to produce queries, and maintenance of an audit file which includes all database modifications) will be followed to ensure accurate data. Clinical personnel will review all data listings for outliers, data inconsistencies, and spelling errors.

During the course of the study, a study monitor (CRA) will make site visits to review protocol compliance, compare eCRFs against individual subject's medical records, assess drug accountability, and ensure that the study is being conducted according to pertinent regulatory requirements.

Electronic CRF entries will be verified with source documentation. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained. Checking the eCRFs for completeness, clarity and cross checking with source documents is required to monitor the progress of the study. Direct access to source data is also required for inspections and audits and will be carried out giving due consideration to data protection and medical confidentiality.

14.2 Record Keeping

Study records and source documents need to be preserved for at least 15 years after the completion or discontinuation of/withdrawal from the study or 2 years after the last approval of a marketing application of HU6 in an ICH region, whichever is the longest time period. The sponsor will be notified prior to the planned destruction of any study related source documents.

15 FINANCING AND INSURANCE

Financial aspects of the study are addressed in a separate clinical study agreement.

The Investigator is required to have adequate current insurance to cover claims for negligence and/or malpractice. The Sponsor will provide insurance coverage for the clinical study as required by national regulations.

16 USE OF DATA AND PUBLICATION POLICY

Both the use of data and the publication policy are detailed within the clinical study agreement.

The Investigator should be aware that intellectual property rights (and related matters) generated by the Investigator and others performing the clinical study will be subject to the terms of a clinical study agreement that will be agreed between the Institution and the Sponsor or their designee. With respect to such rights, the Sponsor or their designee will solely own all right and interest in any materials, data and

intellectual property rights developed by the Investigator and others performing the clinical study described in this protocol, subject to the terms of any such agreement. In order to facilitate such ownership, the Investigator will be required to assign all such inventions either to the Institution where the study is conducted or directly to the Sponsor or their designee, as will be set forth in the clinical study agreement. This agreement will not preclude the reporting of any required data to Regulatory Authorities.

17 REFERENCES

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18 APPENDICES

Appendix A: AUDIT Questions and Scoring System

Questions	0 Points	1 Point	2 Points	3 Points	4 Points
1. How often do you have a drink containing alcohol?	Never	Monthly or less	2-4 times a month	2-3 times a week	4 or more times a week
2. How many drinks containing alcohol do you have on a typical day when you are drinking?	1 or 2	3 or 4	5 or 6	7-9	10 or more
3. How often do you have 6 or more drinks on 1 occasion?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
4. How often during the past year have you found that you were not able to stop drinking once you had started?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
5. How often during the past year have you failed to do what was normally expected of you because of drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
6. How often during the past year have you needed a first drink in the morning to get yourself	Never	Less than monthly	Monthly	Weekly	Daily or almost daily

Questions	0 Points	1 Point	2 Points	3 Points	4 Points
going after a heavy drinking session?					
7. How often during the past year have you had a feeling of guilt or remorse after drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
8. How often during the past year have you been unable to remember what happened the night before because you had been drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
9. Have you or has someone else been injured as a result of your drinking?	No		Yes, but not in the past year		Yes, during the past year
10. Has a relative, friend, or a doctor or other health care worker been concerned about your drinking or suggested you cut down?	No		Yes, but not in the past year		Yes, during the past year

Appendix B: Drugs with Known Risk of Torsade des Pointes

Generic Name	Brand Names (Partial List)	Drug Class	Therapeutic Use	Risk Category	Route
Aclarubicin *(Only on Non US Market)*	Aclacin, Aclacinomycine, Aclacinon, Aclaplastin, Jaclacin	Anti-cancer	Cancer		injection
Amiodarone	Cordarone, Pacerone, Nexterone	Antiarrhythmic	Arrhythmia		oral, injection
Anagrelide	Agrylin, Xagrid	Phosphodiesterase 3inhibitor	Thrombocythemia		oral
Arsenic trioxide	Trisenox	Anti-cancer	Cancer (leukemia)		injection
Astemizole *(Removed from US Market)*	Hismanal	Antihistamine	Allergic rhinitis		oral
Azithromycin	Zithromax, Zmax	Antibiotic	Bacterial infection		oral, injection
Bepridil	Vascor	Antianginal	Angina Pectoris (heart pain)		oral
Cesium Chloride	Energy Catalyst	Toxin	Alternative therapy cancer		oral, injection
Chloroquine	Aralen	Antimalarial	Malaria		oral
Chlorpromazine	Thorazine, Largactil, Megaphen	Antipsychotic / Antiemetic	Nausea, Schizophrenia, many others		oral, injection, suppository
Chlorprothixene *(Only on Non US Market)*	Truxal	Antipsychotic	Schizophrenia		oral, injection
Cilostazol	Pletal	Phosphodiesterase 3inhibitor	Intermittent claudication		oral
Ciprofloxacin	Cipro, Cipro-XR, Neofloxin	Antibiotic	Bacterial infection		oral, injection
Cisapride *(Removed from US Market)*	Propulsid	GI stimulant	Increase GI motility		oral
Citalopram	Celexa, Cipramil	Antidepressant, SSRI	Depression		oral
Clarithromycin	Biaxin, Prevpac	Antibiotic	Bacterial infection		oral,

Generic Name	Brand Names (Partial List)	Drug Class	Therapeutic Use	Risk Category	Route
					inhaled
Cocaine	Cocaine	Local anesthetic	Anesthesia (topical)		oral, nasal
Disopyramide	Norpace	Antiarrhythmic	Arrhythmia		oral, injection
Dofetilide	Tikosyn	Antiarrhythmic	Arrhythmia		oral
Domperidone *(Only on Non US Market)*	Motilium, Motillium, Motinorm Costi, Nomit	Antiemetic	Nausea, vomiting		oral, injection, suppository
Donepezil	Aricept	Cholinesterase inhibitor	Dementia (Alzheimer's Disease)		oral
Dronedarone	Multaq	Antiarrhythmic	Arrhythmia		oral
Droperidol	Inapsine, Droleptan, Dridol, Xomolix	Antipsychotic / Antiemetic	Anesthesia (adjunct), nausea		injection
Erythromycin	E.E.S., Robimycin, EMycin, Erymax, Ery-Tab, Eryc Ranbaxy, Erypar, Eryped, Erythrocin Stearate Filmtab, Erythrocot, E-Base, Erythroped, Ilosone, MY-E, Pediamycin, Abboticin, Abboticin-ES, Erycin, PCE Dispertab, Stiemycine, Acnasol, Tiloryth	Antibiotic	Bacterial infection, increase GImotility		oral, injection
Escitalopram	Cipralex, Lexapro, Nexito, Anxiset-E, Exodus, Esto, Seroplex, Elicea, Lexamil, Lexam, Entact, Losita, Reposil, Animaxen, Esitalo, Lexamil	Antidepressant, SSRI	Depression (major), anxietydisorders		oral
Flecainide	Tambocor, Almarytm, Apocard, Ecrinal, Flécaine	Antiarrhythmic	Arrhythmia		oral
Fluconazole	Diflucan, Trican	Antifungal	Fungal infection		oral, injection
Gatifloxacin *(Removed from US Market)*	Tequin	Antibiotic	Bacterial infection		oral, injection
Grepafloxacin *(Removed from US Market)*	Raxar	Antibiotic	Bacterial infection		oral
Halofantrine *(Only on Non US Market)*	Halfan	Antimalarial	Malaria		oral

Generic Name	Brand Names (Partial List)	Drug Class	Therapeutic Use	Risk Category	Route
Haloperidol	Haldol, Aloperidin, Bioperidolo, Brotopon, Dozic, Duraperidol, Einalon S, Eukystol, Halosten, Keselan, Linton, Peluces, Serenace, Serenase, Sigaperidol	Antipsychotic	Schizophrenia, agitation		oral, injection
Hydroquinidine (Dihydroquinidine) *(Only on Non US Market)*	Serecor	Antiarrhythmic	Arrhythmia		oral
Hydroxychloroquine	Plaquenil, Quineprox	Antimalarial, Anti-inflammatory	Malaria, SLE, rheumatoid arthritis		oral
Ibogaine *(Only on Non US Market)*		Psychedelic	Narcotic addiction, unproven		oral
Ibutilide	Corvert	Antiarrhythmic	Arrhythmia		injection
Levofloxacin	Levaquin, Tavanic	Antibiotic	Bacterial infection		oral, injection
Levomepromazine (Methotrimeprazine) *(Only on Non US Market)*	Nosinan, Nozinan, Levoprome	Antipsychotic	Schizophrenia		oral, injection
Levomethadyl acetate *(Removed from US Market)*	Orlaam	Opioid agonist	Narcotic dependence		oral
Levosulpiride *(Only on Non US Market)*	Lesuride, Levazeo, Enliva	Antipsychotic	Schizophrenia		oral, injection
Meglumine antimoniate *(Only on Non US Market)*	Glucantime	Antiparasitic	Leishmaniasis		injection
Mesoridazine *(Removed from US Market)*	Serentil	Antipsychotic	Schizophrenia		oral
Methadone	Dolophine, Symoron, Amidone, Methadose, Physeptone, Heptadon	Opioid agonist	Narcotic dependence, pain		oral, injection
Moxifloxacin	Avelox, Avalox, Avelon	Antibiotic	Bacterial infection		oral, injection
Nifekalant	Shinbit	Antiarrhythmic	Arrhythmia		injection

Generic Name	Brand Names (Partial List)	Drug Class	Therapeutic Use	Risk Category	Route
(Only on Non US Market)					
Ondansetron	Zofran, Anset, Ondemet, Zuplenz, Emetron, Ondavell, Emeset, Ondisolv, Setronax	Antiemetic	Nausea, vomiting		oral, injection, suppository
Oxaliplatin	Eloxatin	Anti-cancer	Cancer		injection
Papaverine HCl (Intra-coronary)		Vasodilator, Coronary	Diagnostic adjunct		injection
Pentamidine	Pentam	Antifungal	Fungal infection (Pneumocystis pneumonia)		injection, inhaled
Pimozide	Orap	Antipsychotic	Tourette's Disorder		oral
Probucol *(Removed from US Market)*	Lorelco	Antilipemic	Hypercholesterolemia		oral
Procainamide	Pronestyl, Procan	Antiarrhythmic	Arrhythmia		injection
Propofol	Diprivan, Propoven	Anesthetic, general	Anesthesia		injection
Quinidine	Quinaglute, Duraquin, Quinact, Quinidex, Cin-Quin, Quinora	Antiarrhythmic	Arrhythmia		oral, injection
Roxithromycin *(Only on Non US Market)*	Rulide, Xthrocin, Roxl-150, Roxo, Surlid, Rulide, Biaxsig, Roxar, Roximycinv, Roxomycin, Rulid, Tirabicin, Coroxin	Antibiotic	Bacterial infection		oral
Sertindole *(Only on Non US Market)*	Serdolect, Serlect	Antipsychotic, atypical	Schizophrenia, anxiety		oral
Sevoflurane	Ultane, Sojourn	Anesthetic, general	Anesthesia		inhaled
Sotalol	Betapace, Sotalex, Sotacor	Antiarrhythmic	Arrhythmia		oral
Sparfloxacin *(Removed from US Market)*	Zagam	Antibiotic	Bacterial infection		oral
Sulpiride *(Only on Non US Market)*	Dogmatil, Dolmatil, Eglonyl, Espiride, Modal, Sulpor	Antipsychotic, atypical	Schizophrenia		oral, inhaled

Generic Name	Brand Names (Partial List)	Drug Class	Therapeutic Use	Risk Category	Route
Sultopride *(Only on Non US Market)*	Barnetil, Barnotil, Topral	Antipsychotic, atypical	Schizophrenia	⚠ CLQT	oral, injection
Terfenadine *(Removed from US Market)*	Seldane	Antihistamine	Allergic rhinitis	⚠ CLQT	oral
Terlipressin *(Only on Non US Market)*	Teripress, Glypressin, Terlipin,Remestyp, Tresil, Teriss	Vasoconstrictor	Septic shock	⚠ CLQT	injection
Terodiline *(Only on Non US Market)*	Micturin, Mictrol	Muscle relaxant	Bladder spasm	⚠ CLQT	oral
Thioridazine	Mellaril, Novoridazine, Thioril	Antipsychotic	Schizophrenia	⚠ CLQT	oral
Vandetanib	Caprelsa	Anti-cancer	Cancer (thyroid)	⚠ CLQT	oral

⚠ **Known Risk of TdP -** Substantial evidence supports the conclusion that these drugs prolong the QT interval AND are clearlyassociated with a risk of TdP, even when taken as directed in official labeling.

CLQT **Drugs to Avoid in Congenital Long QT -** Substantial evidence supports the conclusion that these drugs pose a risk of TdP for patients with congenital long QT. Drugs on this list include those in the above three risk categories and other drugs that do not prolongthe QT interval per se but they have a theoretical risk of causing arrhythmia that is based on their known stimulant actions on the heart.

Last revision: 17Dec2020
https://crediblemeds.org/

Appendix C: Algorithm for Monitoring and Management of Possible Hepatocellular DILI in Phase 2 or 3 NASH Clinical Studies

Treatment-emergent ALT	Treatment-emergent Total Bilirubin	Liver-related Symptoms	Action[b]
Normal/near normal baseline[a]: ALT ≥5× ULN Elevated baseline[a]: ALT ≥3× baseline or ≥300 U/L (whichever occurs first)	Normal Patients with Gilbert's syndrome: No change in baseline TBL	None	Repeat ALT, AST, ALP, TBL, in 2-5 days. Follow-up for symptoms. Initiate evaluation for other etiologies of abnormal liver tests.
Normal/near normal baseline[a]: ALT ≥3× ULN Elevated baseline[a]: ALT ≥2× baseline or ≥300 U/L (whichever occurs first)	Normal Patients with Gilbert's syndrome: No change in baseline TBL	Severe fatigue, nausea, vomiting, right upper quadrant pain	Repeat ALT, AST, ALP, TBL, in 2-5 days. Follow-up for symptoms. Initiate evaluation for other etiologies of abnormal liver tests.
Normal/near normal baseline[a]: ALT ≥8× ULN Elevated baseline[a]: ALT ≥5× baseline or ≥500 U/L (whichever occurs first)	Normal Patients with Gilbert's syndrome: No change in baseline TBL	None	Interrupt study drug. Initiate close monitoring and workup for competing etiologies. Study drug can be restarted only if another etiology is identified and liver enzymes return to baseline.
Normal/near normal baseline[a]: ALT ≥3 ULN Elevated baseline[a]: ALT ≥2× baseline or ≥300 U/L (whichever occurs first)	TBL ≥2× ULN Patients with Gilbert's syndrome: Doubling of direct bilirubin or increased INR to >1.5	None	Interrupt study drug. Initiate close monitoring and workup for competing etiologies. Study drug can be restarted only if another etiology is identified, and liver enzymes return to baseline.
Normal/near normal baseline[a]:“ ALT ≥5 ULN Elevated baseline[a]: ALT ≥3× baseline or ≥300 U/L (whichever occurs first)	Normal or elevated	Severe fatigue, nausea, vomiting, right upper quadrant pain	Interrupt study drug. Initiate close monitoring and workup for competing etiologies. Study drug can be restarted only if another etiology is identified, and liver enzymes return to baseline.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DILI, drug-induced liver injury; INR, international normalized ratio; TBL, total bilirubin, ULN, upper limit of normal.

a. Baseline ALT is derived from an average of two pre-treatment ALT measurements at least 2 weeks apart. Elevated baseline is defined as ALT ≥1.5×ULN. In patients with a sizable stable decrease in ALT (>50% of the baseline value) during treatment, a new baseline, corresponding to the ALT nadir, should be established on an individual basis for subsequent determination of a DILI signal.

b. The actions of close observation, monitoring, and drug interruption often overlap. Occasionally, workup is initiated after drug interruption.

For further details, please see full Consensus and Recommendation list in Regev A et al., 2019.