Clinical Study Protocol NS-WM-01

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE EFFECT OF TWO FIXED-DOSE LEUCINE AND SILDENAFIL COMBINATIONS (NS-0300) OR TWO FIXED-DOSE LEUCINE, SILDENAFIL AND METFORMIN COMBINATIONS (NS-0200) VERSUS PLACEBO ON BODY WEIGHT IN OBESITY

Phase 2

Original Protocol: August 29, 2017
Amendment #1: October 26, 2017
Amendment #2: November 16, 2017
Amendment #3: December 4, 2017

IND Number: **135732**

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Protocol approved by:

Orville Kolterman, MD
Medical Monitor
Chief Medical Officer

Date

December 4, 2017

PROTOCOL SUMMARY

SPONSOR: NuSirt Sciences, Inc. (D.B.A NuSirt Biopharma)

STUDY TITLE: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE EFFECT OF TWO FIXED-DOSE LEUCINE AND SILDENAFIL COMBINATIONS (NS-0300) OR TWO FIXED-DOSE LEUCINE, SILDENAFIL AND METFORMIN COMBINATIONS (NS-0200) VERSUS PLACEBO ON BODY WEIGHT IN OBESITY

STUDY PHASE: 2

PRIMARY OBJECTIVE

- To evaluate the percentage body weight change in subjects from Baseline/Visit 2 (Day1) to Study Termination/Visit 8 (Day 168/Week 24) receiving two fixed-dose combinations of leucine and sildenafil or two fixed-dose combinations of leucine, sildenafil and metformin compared to placebo

SECONDARY OBJECTIVES

To evaluate the effects of receiving two fixed-dose combinations of leucine and sildenafil or two fixed-dose combinations of leucine, sildenafil and metformin compared to placebo in obese patients on the following parameters:

- Change in absolute body weight from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Change in percentage of patients with ≥5% body weight loss from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Change in waist circumference from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Change in blood lipids (total, HDL- and LDL-cholesterol; triglycerides) from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Change in glycemic control (fasting glucose and HbA1c) from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Change in blood pressure (systolic and diastolic) from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Change in inflammatory markers (hsCRP)from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Exploratory endpoint of percentage of patients with ≥10% body weight loss from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)

STUDY DESIGN

This is a 24-week, randomized placebo-controlled, double-blind study designed to evaluate the effect of two fixed-dose combinations (FDC) of leucine and sildenafil or two FDC of leucine, sildenafil and metformin compared to placebo on body weight.

Subjects meeting all inclusion criteria with no exclusion criteria will be randomized to one of five treatment arms:

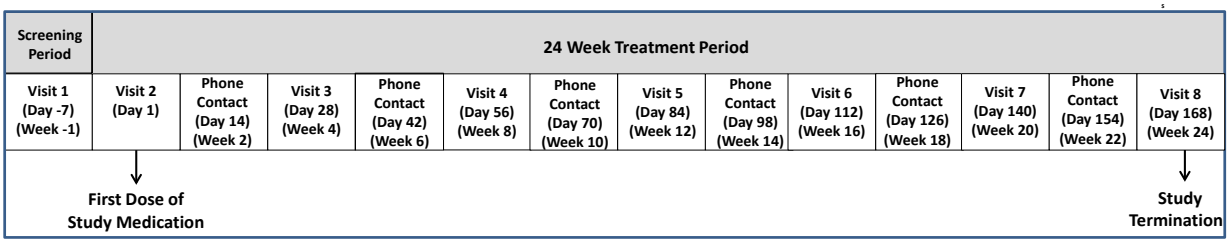
Treatment Arms (Protocol NS-WM-01)

Treatment Arm	Subjects (N) Randomized	Dosing Regimen	Study Medication BID*		
			Leucine	Sildenafil	Metformin
A	50	Placebo	N/A	N/A	N/A
B	50	FDC	1100 mg	1.0mg	N/A
C	50	FDC	1100 mg	4.0 mg	N/A
D	50	FDC	1100 mg	1.0 mg	500 mg
E	50	FDC	1100 mg	4.0 mg	500 mg

FDC = Fixed Dose Combination
*Note: Total daily dose is twice the doses listed in table

The study will include 2 periods: Screening (to determine if inclusion/exclusion criteria are met), and a 24-week, randomized treatment period (Visit 2 to Visit 8) with Visit 2 as the first dose of study medication.

Study Design (Protocol NS-WM-01)



VISIT STRUCTURE AND STUDY DURATION

This study will include a total of 8 visits to the clinical study site: Screening /Visit 1 (Day-7/Week-1), Baseline/Visit 2 (Day 1), Visit 3 (Day 28/Week 4), Visit 4 (Day 56/Week 8), Visit 5 (Day 84/Week 12), Visit 6 (Day 112/Week 16), Visit 7 (Day 140/Week 20) and Study Termination/Visit 8 (Day 168/Week 24). In addition, subjects will be contacted on Day 14/Week 2, Day 42/Week 6, Day 70/Week 10, Day 98/Week 14, Day 126/Week 18 and Day 154/Week 22 to maintain engagement and enhance subject retention.

All study visits should occur within a visit window of ±3 days. Total study duration should not exceed a maximum of 28 weeks.

STUDY POPULATION

A sufficient number of subjects will be exposed to study screening procedures to enroll approximately 250 subjects, a maximum of 70% female, with a goal of having 200 subjects completing all assessments over 24 weeks.

STUDY MEDICATIONS

Subjects will be assigned to one of five treatment groups based on a prospectively defined randomization schedule (see Study Design). Each treatment group will be asked to ingest 3 tablets of blinded study medication 30 minutes before the morning and evening meals.

- **Fixed Dose Combination Arms:** Each dose of study medication will consist of three tablets, two tablets containing either 550mg L-leucine or 550 mg L-leucine/250 mg metformin and one tablet containing either 1.0 mg or 4.0 mg of sildenafil.
- **Placebo Arm:** Each dose of placebo will consist of three tablets, identical in appearance to those used in the two active treatment arms, containing 93.5% Microcrystalline cellulose PH 102, 5.0% Crospovidone XL 10, 1.0% Silica gel (Syloid 244), 0.5% Magnesium stearate I MF3V for the leucine matched placebo and 99.5% Avicel PH200 , 0.5% magnesium stearate (w/w) and Opadry II White coating 3% weight gain for the sildenafil matched placebo.

Study medication will be self-administered by the patient orally 30 minutes before the morning, and evening meals.

EFFICACY ASSESSMENTS

- Percentage change in body weight
- Change in absolute body weight
- Percentage of patients with ≥5% body weight loss
- Change in waist circumference
- Change in lipids:
    - Cholesterol
    - LDL
    - HDL
    - Triglycerides
- Change in glycemic control (fasting glucose and HbA1c)
- Change in blood pressure (systolic and diastolic)
- Change in inflammatory markers (hsCRP)
- Exploratory endpoint of percentage of patients with ≥10% body weight loss

SAFETY ASSESSMENTS

- Adverse events (AE), treatment emergent AEs (TEAEs), and Serious Adverse Event (SAEs) reports
- Vital signs
- Physical examination
- 12-lead ECGs at Screening and Study Termination of study participation
- Clinical chemistry panel, hematology panel, and urinalysis
- Screening β-hCG for females to insure that the subject is not pregnant
- Change in concomitant medications

STATISTICAL CONSIDERATIONS

Analysis Populations:

Enrolled: The Enrolled Population will consist of all subjects who signed the informed consent to participate in the study, and had Screening visit electronic case report forms (eCRFs) collected.

Randomized: The Randomized Population will consist of all Enrolled subjects who were randomized at Baseline/Visit 2 (Day 1)

Intent-To-Treat (ITT): The ITT Population will include all randomized subjects who receive at least one dose of study medication.

Per-Protocol (PP): The PP Population will include all ITT subjects who had adequate exposure to the randomized study medication (>85%) during the 24-week period as defined in the final statistical analysis plan, have completed all study visits and have adequately complied with the protocol as assessed by the investigator and sponsor prior to database lock. The final definition of the PP Population will be documented in an addendum to the prospectively developed statistical analysis plan (SAP) prior to unblinding any data.

Analysis Endpoints:

Primary Efficacy Endpoint: The primary efficacy endpoint is percentage change from Baseline/Visit 2 (Day1) to Study Termination/Visit 8 (Day 168/Week 24) in body weight

Primary Safety and Tolerability Endpoint: The primary safety and tolerability endpoint is the incidence of all treatment-emergent adverse events.

Statistical Analyses:

Descriptive statistics (n, mean, SD, SE, median, min, and max) will be used to summarize values of the primary efficacy endpoint, change in percent body weight from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24) and intermediate study visits as applicable by treatment group. These summaries will be presented for the PP Population with observed data and the ITT Population with Last Observation Carried Forward (LOCF) approach. A mixed-model of analysis of variance (ANCOVA) will be used to analyze change in body weight over the study duration in PP and ITT Populations. The ANCOVA will include treatment group and time as the main effects with baseline weight as the covariate. The LS means, SEs, and the corresponding 95% confidence intervals (CIs) for the changes from baseline to Week 24 will be derived from the model for each treatment. Each of the fixed-dose combinations of leucine, sildenafil, and/or metformin treatment groups (Treatment B, C, D and E) will be compared to placebo group (Treatment A), and the LS means for the treatment difference (Treatment B, C D or E minus Treatment A), the SEs, the associated 95% CIs, and the p-values will be computed.

SAMPLE SIZE

A sufficient number of individuals will be screened to enroll at least 250 subjects (~50/treatment arm). With an assumption of 20% dropout rate, approximately 200 subjects (~40/treatment arm) are expected to complete treatment through Study Termination/Visit 8 (Day 168/Week 24).

Using a two-sided, two-sample comparison (t-test) of means at the alpha=0.05 level of significance, sample sizes of 23 subjects per treatment will provide approximately 90% power to detect a mean treatment difference of 3 kg body weight (~3.0-3.3% weight loss over 24 weeks), assuming a common standard deviation of 25%. However, detection of significant changes in obesity co-morbidities require a higher sample size; detection of a 6 mm Hg change in blood pressure will require a sample size of 40 subjects per treatment group at 80% power; accordingly, the sample size has been increased to 40/treatment arm to enable assessment of this secondary endpoint. These assumptions of effect size and standard deviation are based on data derived from the previous clinical trial of NS-0200 (NS-0200-01) and represent the anticipated treatment effect of effective fixed-dose combinations of leucine and sildenafil, with or without metformin, compared to placebo.

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AE	adverse event
ALT	alanine transaminase
AMPK	5' adenosine monophosphate-activated protein kinase
AST	aspartate transaminase
β–hCG	beta subunit of human chorionic gonadotropin
BID	twice daily
BMI	body mass index
CI	confidence interval
CFR	Code of Federal Regulations
CPK	creatine phosphokinase
CK-MB	creatine kinase-MB
CRF	case report form
CRO	contract research organization
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
FDA	Food and Drug Administration
FDC	fixed dose combination
mg	milligram
GCP	Good Clinical Practices
hr	hour(s)
HbA1c	glycosylated hemoglobin
HDL-C	high density lipoprotein-cholesterol
HIPAA	Health Insurance Portability and Accountability Act
hs-CRP	High sensitivty C-reactive protein
ICF	Informed Consent Form
IND	Investigational New Drug
IRB	Institutional Review Board
LDL-C	low density lipoprotein-cholesterol
LOCF	last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
min	minute(s)
NDA	New Drug Application
SAE	serious adverse event
SD	standard deviation
Sirt 1	NAD-dependent deacetylase sirtuin 1
TEAE	treatment-emergent adverse event

1 INTRODUCTION AND BACKGROUND

Global obesity prevalence has doubled over the past three decades, and the obesity epidemic now represents a significant public health risk in the US and other developed countries as well as in the developing world. In the US, >35% of the adult population and 17% of the pediatric and adolescent populations are obese, and ~70% of the population is overweight or obese [1,2]. Obesity is associated with significant mortality risk, as shown by a 9-13 year reduction in lifespan and >300,000 obesity-related deaths annually in the US [3–7]. Further, obesity increases the risk of co-morbidities, including cardiovascular disease, type 2 diabetes, hypertension, dyslipidemia, obstructive sleep apnea, and some cancers [4–8]. The risk of obesity-related co-morbidities increases with increasing body mass index (BMI); conversely, even modest reduction in BMI resulting from a 5-10% weight loss reduces the risk and severity of these co-morbidities [9,10].

Lifestyle intervention focused on reducing caloric intake and increasing caloric expenditure are the first-line approach to obesity management; however, patient adherence is generally poor and obesity control through lifestyle intervention alone rarely exhibits sustained success. Accordingly, pharmacotherapy is an important adjunct to diet and exercise to achieve sustained weight reduction. The proposed fixed dose combination of the amino acid leucine and two well characterized agents with established good safety profiles is expected to modulate energy metabolism and facilitate sustained weight loss.

AMPK and Sirt1 are well known regulators of lipid and energy metabolism. Activation of Sirt1 and AMPK stimulates mitochondrial biogenesis and fat oxidation with concomitant suppression of lipid synthesis and storage [11–16]. NuSirt Biopharma has demonstrated that L-leucine has a unique role as an activator of the Sirt1/AMPK pathway, serving as a partial mimetic of caloric restriction, and thereby modulates Sirt1-dependent lipid and energy metabolism [17–19]. NuSirt has shown that addition of L-leucine to metformin (fixed dose combination, NS-0100) results in a novel synergistic interaction that enables significant dose reduction of metformin with no loss of anti-diabetic efficacy (IND# 121112) and that a triple combination of L-leucine with low-dose metformin and sildenafil (NS-0200) regresses steatohepatitis in preclinical studies [19,20] and reduces hepatic fat in non-alcoholic fatty liver disease patients with elevated liver enzymes (IND #126214). Further, data from the Phase 2A trial conducted under this IND also provided a robust weight loss signal accompanied by decreases in obesity associated co-morbidities. This finding is supported by both mechanism of action and preclinical studies demonstrating reductions in adiposity in a rodent obesity model.

NuSirt also has preclinical data which suggests that metformin may not be a necessary component to achieve weight loss; we therefore will evaluate a related fixed dose combination of leucine and sildenafil in the absence of metformin (NS-0300) in this study. The superior combination will then be utilized for further development.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this study is:

- To evaluate the percentage change in weight in subjects from : Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24) receiving two fixed-dose

combinations of leucine and sildenafil or two fixed-dose combinations of leucine, sildenafil and metformin compared to placebo

2.2 Secondary Objectives

The secondary objectives of this study are to evaluate the effects of two fixed-dose combinations of leucine and sildenafil or two fixed-dose combinations of leucine, sildenafil and metformin compared to placebo in obese on the following parameters:

- Absolute body weight from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Percentage of patients with ≥5% body weight loss from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Change in waist circumference from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Change in blood lipids (cholesterol, LDL, HDL, triglycerides) from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Change in fasting glucose from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Change in HbA1cfrom Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Change in Blood pressure (diastolic and systolic) from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Change in hsCRP from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- Safety and tolerability
- Exploratory endpoint of percentage of patients with ≥10% body weight loss from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)

3 STUDY DESIGN

3.1 Design Description

This is a randomized, 24-week, placebo-controlled, double-blind study to evaluate the effects of two fixed-dose combinations of leucine and sildenafil or two fixed-dose combinations of leucine, sildenafil and metformin compared to placebo.

Subjects meeting all inclusion criteria with no exclusion criteria will be randomized to one of five treatment arms (**Table 1**).

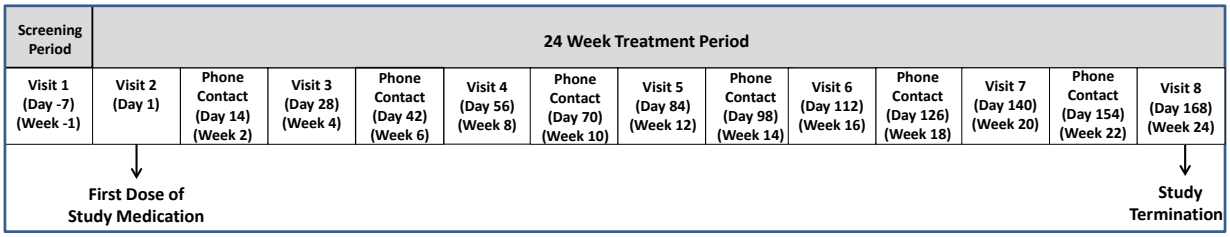
Table 1: Treatment Arms (Protocol NS-WM-01)

Treatment Arm	Subjects (N) Randomized	Dosing Regimen	Study Medication Dosed Twice/Day[1]		
			Leucine	Sildenafil	Metformin
A	50	Placebo	N/A	N/A	N/A
B	50	FDC	1100 mg	1 mg	N/A
C	50	FDC	1100 mg	4 mg	N/A
D	50	FDC	1100 mg	1 mg	500mg
E	50	FDC	1100 mg	4 mg	500mg

FDC = Fixed Dose Combination
*Note: The total daily dose is twice that listed for each compound

The study design is depicted in **Figure 1**. The study will include a total of 2 periods: Screening (including a 7 day period for lab results) and a 24-week treatment period (Day 1 to Day 168) with the first dose of study medication being administered on Day 1.

Figure 1: Study Design (Protocol NS-WM-01)



3.2 Visit Structure and Study Duration

This study will include a total of 8 visits to the clinical study site (**Figure 1**) as follows: Screening/Visit 1 (Day-7/Week-1), Baseline/Visit 2 (Day 1), Visit 3 (Day 28/ Week 4), Visit 4 (Day 56/Week 8), Visit 5 (Day 84/Week 12), Visit 6 (Day 112/Week 16), Visit 7 (Day 140/Week 20) and Study Termination/Visit 8 (Day 168/Week 24). In addition, subjects will receive six telephone contacts on Day 14/Week 2, Day 42/Week 6, Day 70/Week 10, Day98/Week 14, Day126/ Week 18 and Day 154/Week 22.

For all subjects, Visit 1 should be Day -7 ± 3 days relative to Baseline/Visit 2 (Day 1). During the 24-week treatment period, the visit window will not exceed ± 3 days for Visit 3 (Day 28/ Week 4), Visit 4 (Day 56/Week 8), Visit 5 (Day 84/Week 12), Visit 6 (Day 112/Week 16), Visit 7 (Day 140/Week 20) and Study Termination/Visit 8 (Day 168/Week 24). Total study duration should not exceed a maximum of 28 weeks.

4 STUDY POPULATION

4.1 Population to Be Studied

A sufficient number of obese subjects will be exposed to study screening procedures to enroll approximately 250 subjects, with a maximum of 70% female, with a goal of having at least 200 subjects completing all assessments over the 24-week assessment period. The definition of obesity is a BMI ≥30 kg/m^2. For purposes of this study we will not include individuals with a BMI >45 kg/m^2.

4.2 Inclusion Criteria

Individuals meeting all of the following criteria will be considered for admission to the study:

1. Age ≥18 and ≤65 at study entry.
2. Is male, or female and, if female, meets all of the following criteria:
   a. Not breastfeeding
   b. Post-menopausal or negative serum pregnancy test result (human chorionic gonadotropin, beta subunit [β-hCG]) at Screening/Visit 1 (Day-7/Week-1) (not required for hysterectomized females)
   c. If of childbearing potential (including peri-menopausal women who have had a menstrual period within one year) must practice and be willing to continue to practice appropriate birth control (defined as a method which results in a low failure rate, i.e., less than 1% per year, when used consistently and correctly, such as double barrier methods [male condom with spermicide, with or without cervical cap or diaphragm], implants, injectables, oral contraceptives [must have been using for at least the last 3 months], some intrauterine contraceptive devices, tubal ligation, or in an established relationship with a vasectomized or same sex partner) during the entire duration of the study
3. Stable body weight (±5%) and health over the last 3 months.
4. Has a BMI between 30 kg/m^2 and 45 kg/m^2
5. Stable diet within the last three months
6. Subjects on antidepressants, excluding those listed in the exclusion criteria, must have been on a stable dose regiment for at least 3 months prior to study enrollment and maintain stable dose during the study
7. Clinical laboratory tests (hematology, clinical chemistry, and urinalysis) either normal, not clinically significant or with abnormalities consistent with obesity
8. Is able to read, understand, and sign the informed consent forms (ICF) and, when applicable, an authorization to use and disclose protected health information form (consistent with Health Insurance Portability and Accountability Act of 1996 [HIPAA] legislation), communicate with the investigator, and understand and comply with protocol requirements.

4.3 Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from the study.

1. Diagnosis of diabetes or on a diabetes medication
2. HbA1c ≥6.5% at Screening/Visit 1 (Day-7/Week-1)
3. Severe renal impairment (eGFR < 45 mg/mL/1.73 m2) and/or patients with acute or chronic metabolic acidosis. Acidosis is defined as >25mmol/L computed without K. Normal is 8-16, but acidosis is >25
4. Use of any of the following medications in the eight weeks prior to entering screening for study participation and during the study:
    a. Use of any anti-diabetes medication including metformin and any combination drug that contains metformin
    b. Sildenafil
    c. Tadalafil
    d. Vardenafil
    e. OCT2/MATE inhibitors (e.g. cimetidine, quinidine, and pyrimethamine)
    f. Riociguat (guanylate cyclase stimulant)
    g. Alpha blockers
    h. Nitrates (e.g. oral nitrates, sublingual nitroglycerine and nitroglycerine patches)
    i. Potent CYP3A4 inhibitors (e.g. clarithromycin, telithromycin, nefazodone, itraconazole, ketoconazole, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranavir)
    j. All antihypertensive medications
    k. Medications associated with weight changes
        - Drugs approved for the treatment of obesity
        - Cyproheptadine or medroxyprogesterone
        - Atypical anti-psychotic drugs
        - Tricyclic antidepressants
        - Lithium, MAO's, glucocorticoids
        - SSRI's or SNRI's
        - Antiepileptic drugs
        - Systemic corticosteroids
        - Stimulants e.g. amphetamines
    l. Any dietary supplement that is labeled for weight management or maintenance of healthy weight
5. Diagnosis or evidence of eating disorders
6. ≥ 5% weight change in the last 3 months

7. Subjects who have had bariatric surgery
8. An infection requiring antibiotic treatment within the last 30 days
9. Any chronic inflammatory disease, e.g. inflammatory bowel disease rheumatoid arthritis, vasculitis, systemic lupus erythematosus
10. Diseases interfering with metabolism (including metabolism of branched chain amino acids) and/or ingestive behavior (myxedema, Cushing's disease, diabetes, schizophrenia, major psychoses, maple syrup urine disease, unmanaged depression etc.)
11. History of alcohol abuse (defined ≥ 21 drinks per week for males and > 14 drinks per week for females), within the past 3 months or failure on urinary drug screen
12. History of substance abuse (including alcohol abuse as defined above) in the past 12 months or a positive screen for drugs (opioids without a prescription), including marijuana, of abuse or alcohol at screening.
13. Has received any investigational drug within 3 months of Screening.
14. Has donated blood within 3 months before Screening or is planning to donate blood during the study (due to HbA1c reading at screening)
15. Other medical conditions that may diminish life expectancy to <2 years, including known cancers
16. Have been diagnosed with metastatic carcinomas in the last 5 years
17. Has known allergies or hypersensitivity to metformin, sildenafil or leucine
18. Have suffered myocardial infarction, stroke, arrhythmia in the last 6 months
19. Resting hypotension (BP <90/50 mmHg) or severe hypertension (BP >170/110 mmHg) If both or one of the parameters for diastolic or systolic BP is meet
20. Cardiac failure or coronary artery disease causing unstable angina
21. History or evidence at screening of left ventricular outflow obstruction (e.g., aortic stenosis, idiopathic hypertrophic subaortic stenosis) and those with severely impaired autonomic control of blood pressure
22. At risk for priapism due to anatomical deformation of the penis (e.g., angulation, cavernosal fibrosis, Peyronie's disease) or other conditions that predispose to priapism (e.g., sickle cell anemia, multiple myeloma, or leukemia)
23. Clinical evidence of hepatic impairment and/or ALT/AST >5X ULN
24. Is an immediate family member (spouse, parent, child, or sibling; biological or legally adopted) of personnel directly affiliated with the study at the clinical study site, or NuSirt Biopharma.
25. Is employed by NuSirt Biopharma (defined as an employee, temporary contract worker, or designee responsible for the conduct of the study).

4.4 Removal of Subjects from Study Participation

Participants are to be removed from the study if any of the following conditions are met after study entry:

- Withdrawal of Consent - Subject wishes to withdraw from the study as outlined in the informed consent. All subjects reserved the right to withdraw from the study without prejudice to themselves or their future medical care. This implies a direct request to withdraw from the study and a refusal of further contact.
- Adverse Event - Subject experiences an adverse event, which in the investigator's opinion necessitates withdrawal from the study.
- Investigator Decision - Investigator assessment that it is in the subject's best interest to terminate participation.
- Protocol Violation – Includes subject non-compliance, documented pregnancy, study entry violation, and/or start of an unacceptable concomitant medication (listed in section 4.3).
- Lost To Follow-Up - Subject fails to return for study visits and cannot be reached with reasonable attempts. At the close of the study one final attempt to contact the subject will be made to attempt to determine the subjects well-being.
- Administrative Reason – Includes discontinuation of the study protocol by the sponsor, the Food and Drug Administration (FDA), or other regulatory authority, and/or discontinuation of the study site's participation in the study protocol.
- Pregnancy

Any withdrawal from the study will be fully documented on the subject's eCRF and in the subject's study record at the site. The documentation will include reasons for the withdrawal and details of any sequelae (followed through until the symptoms resolved or returned to baseline levels) if the reason was an adverse event.

Every effort will be made to conduct all protocol specified procedures and examinations required to complete the study. Optimally, all procedures should be performed within 24 to 48 hours of termination and, if possible, prior to initiating new therapies. Diligent attempts will be made to follow through endpoint assessment those patients who do not withdraw consent and/or are not lost to follow up.

Subjects who discontinue from the study after randomization and prior to completion of Study Termination /Visit 8 will be considered early withdrawals and, if enrollment is still open, enrollment may be expanded to ensure a full complement of evaluable subjects.

4.5 Subject Restrictions

Once screened and qualified for entry, subjects will be instructed as follows:

- Take no new prescription or over-the-counter medications without prior notification of the investigator. For restrictions on concomitant medications, refer to **Section 4.3**
- Remain on their usual diet and exercise regimen throughout the study

- Fast overnight for at least 10 hours (no food or beverage except water) prior to each visit
- Do not donate blood for the duration of the study

The sponsor should be contacted if the investigator is informed of any restriction violations. The sponsor will decide whether a subject with restriction violations will be granted a deviation and allowed to continue study participation.

4.6 Concomitant Medications

Subjects must follow the medication restrictions outlined in the inclusion and exclusion criteria (Section 4.3) during the study. Dosages for certain concomitant medications should be maintained constant during the study (Section 4.2 and 4.3) unless instructed otherwise by the investigator or a treating physician. Any change in regimen for any concomitant medication, including restricted concomitant medications, must be reported to the sponsor and recorded in the eCRF.

The sponsor should be contacted if the investigator is informed of consistent concomitant medication restriction violations. The sponsor will decide whether the subject will be allowed to continue study participation.

5 STUDY MEDICATIONS

Study medications administered in this protocol include either placebo or one of four combinations of leucine, sildenafil and/or metformin (See Section 5.1). All drugs will be provided as tablets with identical appearance to double-blind the medications to subjects and study personnel. Study medication is to be kept at ambient temperature at all times. Any deviations will require the drug to be temporarily quarantined and the deviation reported to NuSirt. NuSirt will then make an assessment as to how to proceed.

5.1 Formulation, Packaging, and Storage

Fixed Dose Combination Arms: Each dose of study medication will consist of three tablets, two tablets containing either 550 mg L-leucine alone or 550mg L-leucine in combination with 250 mg of Metformin and one tablet either 1.0 mg or 4.0 mg of sildenafil.

Placebo Arm: Each dose of placebo will consist of three tablets, identical in appearance to those used in the two active treatment arms, containing 93.5% Microcrystalline cellulose PH 102, 5.0% Crospovidone XL 10, 1.0% Silica gel (Syloid 244), 0.5% Magnesium stearate I MF3V for the leucine matched placebo and 99.5% Avicel PH200 , 0.5% magnesium stearate (w/w) and Opadry II White coating 3% weight gain for the sildenafil matched placebo.

5.2 Dispensing of Study Medication

Study materials will be provided to subjects by the investigator, medically qualified sub-investigator named on form FDA 1572, or other qualified study-site personnel. Under no circumstance will the investigator or sub-investigator(s) allow the study medication to be used other than as directed by the protocol or to be administered to any persons other than

subjects who have signed an informed consent and are participating in the study.

Study-site personnel will be responsible for instructing subjects to administer the correct doses of study medication as shown in **Appendix 1**.

Study medication will be provided in blinded, randomized kits. Each kit dispensed will be labeled with a unique package number.

5.3 Dose Administration, Route and Schedule

Study medication will be self-administered by the patient as 3 tablets taken orally BID within 30 minutes prior to the morning and evening meals. Each dose of study medication will consist of 3 tablets, which will be identical in appearance across all treatment groups.

5.4 Randomization Schedule and Blinding Procedures

A randomization schedule will be prepared and not shared with anyone directly involved in study conduct. Subjects will be assigned to one of five treatment groups (**Table 1**) based on the randomization schedule.

Subjects will be assigned a randomization number on Baseline/Visit 2 (Day 1). The randomization numbers will be allocated in strict chronological order. The number will uniquely identify the subject and dictate the assigned, blinded treatment group.

The sponsor personnel involved in study conduct, study staff and subject will be blinded to the identity of study medication during the study. The study medication will be provided to the study centers in a blinded fashion. The study center pharmacist will use the subject's assigned randomization number to ensure that the appropriate blinded study medication is allocated at each visit. The study centers will be provided with sufficient study medication to supply all randomized subjects.

5.5 Drug Accountability

The investigator listed on form FDA 1572 will be responsible for all clinical supplies (study medication) accountability. Upon receipt of all study medication, the contents of each shipment will be verified against the enclosed shipping form. After verification, the form will be signed and dated, a copy will be retained for the investigator's file, and the original will be returned to the sponsor. The sponsor will be notified immediately if any irregularities, discrepancies, or damages are identified.

The investigational materials will be prescribed only by the investigator or sub-investigators. Under no circumstances will the investigator or sub-investigators allow the investigational drug to be used other than as specified in the protocol.

Drug dispensing and returns will be recorded on a sponsor-provided or sponsor-approved drug disposition form, to provide a complete accounting of the receipt, disposition, and return of study medication. At the completion of the study, all remaining used and unused study medication, empty containers, and copies of the completed drug disposition forms will be returned to the sponsor or designee.

6 STUDY METHODS

A detailed schedule of study procedures is summarized in **Appendix 1**.

Prior to all screening and study visits, subjects will be asked to fast overnight for at least 10 hours (i.e., no food or beverage except water). Subjects should report to the clinical study site in the morning of each scheduled visit (ideally between 0700 and 0900 hours). For all study visits after randomization, subjects should refrain from administering the morning dose of study medication prior to the visit, and bring the study medication along to the study-site. The morning dose of study medication will be taken while at the clinic after all study procedures have been completed from one of the two newly dispensed study medication kits.

6.1 Screening (Visit 1/ Day-7/ Week-1)

The following procedures will be performed in order when possible, except for the ICF which must be done before any other items, at Screening:

- The informed consent and HIPAA authorization forms will be signed prior to performing any procedures required by the protocol
- Review of inclusion/exclusion criteria to evaluate subject eligibility
- Complete medical history
- Adverse event review
- Review of concomitant medications
- Vital signs; blood pressure to be done first and using the equipment provided by NuSirt as instructed in section 7.8
- Complete height and weight assessment for BMI calculation
- Complete physical examination
- 12-lead ECG
- Blood sample collection (**fasting samples**) for the following assessments (please follow the laboratory manual for the procedures and order of collection):
  - serum β-hCG test for all female subjects, regardless of menopausal status (not required if subject has had a hysterectomy)
  - Chemistry panel includes glucose, urea nitrogen, creatinine, total protein, albumin, uric acid, total and fractionated bilirubin, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltranspeptidase (GGT), creatine phosphokinase (CPK), creatine kinase-MB (CK-MB, If CPK is elevated), sodium, potassium, chloride, bicarbonate, phosphate, calcium, magnesium
  - Lipid Panel includes total cholesterol, HDL-C, LDL-C and triglycerides
  - Hematology panel (includes red cell count, hemoglobin, hematocrit, white cell count, platelets, differential count, mean cell volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration)
  - HbA1c

- Urine sample collection for the following assessments:
  - urinalysis
  - drug and alcohol screen

Abnormal results of laboratory tests, **not addressed in the Inclusion/Exclusion criteria,** consistent with and associated with obesity will be evaluated on a case by case basis with input from investigator in consultation with the medical monitor. With the exception of abnormal laboratory tests consistent with obesity as mentioned above, individuals will be disqualified if results of any laboratory tests are more than twice the upper limit of normal and clinically significant as judged by the investigator in consultation with the medical monitor.

6.2 Baseline/Visit 2 (Day 1)

Note: All individuals will be counselled at Baseline/Visit 2 (Day 1) that they are not to change eating or exercise routines during the trial.

The following procedures will be completed at Visit 2:

- Review of concomitant medicines
- Adverse event review
- Measurement of vital signs:
  - Blood pressure (sitting systolic and diastolic blood pressure using the equipment provided by NuSirt)
  - Heart rate
- Body weight assessment
- Waist Circumference measurement (using the tape measure provided by NuSirt)
- Randomization to study medication
- Blood sample collection (**fasting samples**) for the following assessments (please follow the laboratory manual for the procedures and order of collection):
  - Chemistry panel
  - Hematology panel
  - Lipid panel
  - hsC-reactive protein (hsCRP)
  - HbA1c
  - Biomarkers (Note: These samples will be archived for future exploratory analyses. See Section 7.10)
- Urine sample collection for urinalysis assessment
- First dose of study medication taken in clinic

- Dispense study medication for 4 weeks (each kit has sufficient study medication for 2 weeks + 1 day)

6.3 Telephone Contact (Day 14/Week 2)

The investigator and/or qualified study-site personnel will contact subjects by telephone on Day 14 to review adverse events and study medication compliance.

6.4 Visit 3 (Day 28/Week 4)

The following procedures will be completed at Visit 3:

- Review of concomitant medicines
- Review of Adverse Events
- Measurement of vital signs:
  - Blood pressure (sitting systolic and diastolic blood pressure using the equipment provided by NuSirt)
  - Heart rate
- Body weight assessment
- Waist Circumference measurement (using the tape measure provided by NuSirt)
- Return of unused study medication
- Review of study medication compliance
- Dispense study medication for next 4 weeks

6.5 Telephone Contact (Day 42/Week 6)

The investigator and/or qualified study-site personnel will contact subjects by telephone on Day 56 to review adverse events and study medication compliance.

6.6 Visit 4 (Day 56/Week 8)

The following procedures will be completed at Visit 4:

- Review of concomitant medicines
- Review of Adverse Events
- Measurement of vital signs:
  - Blood pressure (sitting systolic and diastolic blood pressure using the equipment provided by NuSirt)
  - Heart rate
- Body weight assessment
- Waist Circumference measurement (using the tape measure provided by NuSirt)

- Return of unused study medication
- Review of study medication compliance
- Dispense study medication for next 4 weeks

6.7 Telephone Contact (Day 70/Week 10)

- The investigator and/or qualified study-site personnel will contact subjects by telephone on Day 70 to review adverse events and study medication compliance.

6.8 Visit 5 (Day 84/Week 12)

The following procedures will be completed at Visit 5:

- Review of concomitant medicines
- Review of Adverse Events
- Measurement of vital signs:
  - Blood pressure (sitting systolic and diastolic blood pressure using the equipment provided by NuSirt)
  - Heart rate
- Body weight assessment
- Waist Circumference measurement (using the tape measure provided by NuSirt)
- Blood sample collection (**fasting samples**) for the following assessments (please follow the laboratory manual for the procedures and order of collection):
  - Chemistry panel
  - Hematology panel
  - Lipid panel
  - hsC-reactive protein
  - HbA1c
  - Biomarkers (Note: These samples will be archived for future exploratory analyses. See Section 7.10)
- Return of unused study medication
- Review of study medication compliance
- Dispense study medication for next 4 weeks

6.9 Telephone Contact (Day 98/Week 14)

- The investigator and/or qualified study-site personnel will contact subjects by telephone on Day 98 to review adverse events and study medication compliance.

6.10 Visit 6 (Day 112/Week 16)

The following procedures will be completed at Visit 6:

- Review of concomitant medicines
- Review of Adverse Events
- Measurement of vital signs:
  - Blood pressure (sitting systolic and diastolic blood pressure using the equipment provided by NuSirt)
  - Heart rate
- Body weight assessment
- Waist Circumference measurement (using the tape measure provided by NuSirt)
- Return of unused study medication
- Review of study medication compliance
- Dispense study medication for next 4 weeks

6.11 Telephone Contact (Day 126/Week 18)

- The investigator and/or qualified study-site personnel will contact subjects by telephone on Day 126 to review adverse events and study medication compliance.

6.12 Visit 7 (Day 140/Week 20)

The following procedures will be completed at Visit 7:

- Review of concomitant medicines
- Review of Adverse Events
- Measurement of vital signs:
  - Blood pressure (sitting systolic and diastolic blood pressure using the equipment provided by NuSirt)
  - Heart rate
- Body weight assessment
- Waist Circumference measurement (using the tape measure provided by NuSirt)
- Return of unused study medication
- Review of study medication compliance
- Dispense study medication for next 4 weeks

6.13 Telephone Contact (Day 154/Week 22)

- The investigator and/or qualified study-site personnel will contact subjects by

telephone on Day 154 to review adverse events and study medication compliance.

6.14 Study Termination/Visit 8 (Day 168/Week 24) or Early Termination

The following procedures will be completed at Visit 8 or the termination visit for subjects who leave the study prior to study completion:

- Complete medical history
- Review of concomitant medicines
- Adverse event review
- Measurement of vital signs:
  - Blood pressure(sitting systolic and diastolic blood pressure using the equipment provided by NuSirt)
  - Heart rate
- Body weight assessment
- Waist Circumference measurement (using the tape measure provided by NuSirt)
- Complete physical examination
- 12-lead ECG
- Blood sample collection (**fasting samples**) for the following assessments (please follow the laboratory manual for the procedures and order of collection):
  - Chemistry panel
  - Hematology panel
  - Lipid panel
  - hsC-reactive protein
  - HbA1c
  - Biomarkers (Note: These samples will be archived for future exploratory analyses. See Section 7.10)
- Urine sample collection for urinalysis assessment
- Return of unused study medication
- Review of study medication compliance

6.15 Ethical Safety Considerations

Metformin, on rare occasions, has been associated with lactic acidosis in patients on higher doses than allowed in this study. In addition, these events have usually occurred in patients encountering major medical illnesses superimposed on their diabetes. These issues are addressed in Section 8.1 with an emphasis on early detection.

Treatment with sildenafil at the approved doses of 50 to 100 mg (≥20 times the doses employed

in this study) has been associated with the following rare adverse events:

1. Sudden loss of vision
2. Ringing in the ears
3. Loss of hearing
4. Ischemic cardiovascular events

Should any study participant experience one of these events, study drug is to be held, appropriate medical evaluation arranged and the medical monitor contacted. It will then be the determination of the medical monitor, after discussions with the PI, whether the subject will be removed from the study.

7 EFFICACY ASSESSMENTS

7.1 Percent Change in Body Weight

Body weight will be assessed at Screening/Visit 1 (Day-7/Week-1), Baseline/Visit 2 (Day1), Visit 3 (Day 28/Week4), Visit 4 (Day 56/Week 8), Visit 5 (Day 84/Week 12), Visit 6 (Day 112/Week 16), Visit 7 (Day 140/Week 20) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination. Percentage change is defined as Study Termination/Visit 8 (Day 168/Week 24) or Early Termination weight minus Baseline/Visit 2 (Day1) weight divided by Baseline/Visit 2 (Day1) weight multiplied by 100. Weight measurements will be done without shoes or street clothing; subjects will be weighed in undergarments with gowns (front and back). All scales will have certified calibration and sites will maintain their current calibaration schedule. When possible the same scale that is used for a subject at Screening/Visit 1 (Day-7/Week-1) will be used for all remaining visits for that subject. All weights are to be made and reported in kilograms to the first decimal place.

7.2 Absolute Body Weight

Body weight will be assessed at Screening/Visit 1 (Day-7/Week-1), Baseline/Visit 2 (Day1), Visit 3 (Day 28/Week4), Visit 4 (Day 56/Week 8), Visit 5 (Day 84/Week 12), Visit 6 (Day 112/Week 16), Visit 7 (Day 140/Week 20) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination. All weights are to be made and reported in kilograms to the first decimal place.

7.3 Percentage of patients with ≥5% and ≥10% body weight loss

Body weight will be assessed at Screening/Visit 1 (Day-7/Week-1), Baseline/Visit 2 (Day1), Visit 3 (Day 28/Week4), Visit 4 (Day 56/Week 8), Visit 5 (Day 84/Week 12), Visit 6 (Day 112/Week 16), Visit 7 (Day 140/Week 20) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination. Percentage change is defined as Study Termination/Visit 8 (Day 168/Week 24) or Early Termination weight minus Baseline/Visit 2 (Day1) weight divided by Baseline/Visit 2 (Day1) weight. All weights are to be made and reported in kilograms to the first decimal place.

7.4 Waist Circumference

Waist circumference will be measured at Baseline/Visit 2 (Day1), Visit 3 (Day 28/Week4), Visit 4 (Day 56/Week 8), Visit 5 (Day 84/Week 12), Visit 6 (Day 112/Week 16), Visit 7 (Day 140/Week 20) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination. Waist circumference is measured at the end of several consecutive natural breaths, at a level parallel to the floor, midpoint between the top of the iliac crest and the lower margin of the last palpable rib in the mid axillary line. Measurements are made with a stretch-resistant tape (which will be provided by NuSirt) that is wrapped snugly around the subject, but not to the point that the tape is constricting. The tape is kept level and parallel to the floor at the point of measurement. Subjects will be standing upright during the measurement, with arms relaxed at the side, feet evenly spread apart and body weight evenly distributed. All measurements are to be made and reported in centimeters to the first decimal place.

7.5 Fasting Lipids

Fasting lipid concentrations (total cholesterol, LDL, HDL, triglycerides) will be measured in all subjects from blood samples collected at Screening/Visit 1 (Day-7/Week-1), Baseline/Visit 2 (Day1), Visit 5 (Day 84/Week 12) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination.

7.6 Fasting Glucose

Fasting glucose will be measured in all subjects from blood samples collected at Screening/Visit 1 (Day-7/Week-1), Baseline/Visit 2 (Day1), Visit 5 (Day 84/Week 12) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination.

7.7 HbA1c

HbA1c will be measured in all subjects from blood samples collected at Screening/Visit 1 (Day-7/Week-1), Baseline/Visit 2 (Day1), Visit 5 (Day 84/Week 12) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination.

7.8 Blood Pressure

Blood Pressure will be measured in all subjects at Screening/Visit 1 (Day-7/Week-1), Baseline/Visit 2 (Day1), Visit 3 (Day 28/Week4), Visit 4 (Day 56/Week 8), Visit 5 (Day 84/Week 12), Visit 6 (Day 112/Week 16), Visit 7 (Day 140/Week 20) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination. Blood pressure will be taken using the Omron HEM-907XL provided by NuSirt. Qualified site personnel will program the machine to capture a series of 3 measurements starting with a 5 minute delay followed by a delay of 2 minutes between the first and second reading and the second and third reading. The patient is to be in a seated position with their back resting on the back of the chair, feet flat on the floor and arm resting comfortably approximately at heart level. The appropriate cuff size, cuff placement and wrapping of the cuff on the subjects arm will be

applied following the instruction manual provided with the equipment. Once the patient and equipment is set, site personnel will leave the room for approximately 10 minutes. After the measurements are completed, qualified site personnel will then document the 3 measurements, as well as the average provided by the machine (refer to the Omron HEM-907XL instruction manual for detailed instructions on all the directions listed above).

7.9 hsC-Reactive Protein

hsC-Reactive Protein will be measured in all subjects from blood samples collected at Baseline/Visit 2 (Day1), Visit 5 (Day 84/Week 12) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination.

7.10 Biomarkers

Biomarkers will be measured in all subjects from blood samples collected at Baseline/Visit 2 (Day1), Visit 5 (Day 84/Week 12) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination. Biomarker analysis will not include genetic testing. Biomarker analysis may include but is not limited to assessments of adiponectin, TNFα, IL-6, PAI-1, and a lipidomic panel. Additional non-genetic biomarkers identified subsequent to trial initiation may also be used.

8 SAFETY ASSESSMENTS

Safety will be assessed throughout the study by examination of adverse events, concomitant medications, clinical laboratory evaluations, vital signs, physical examinations, and 12-lead ECGs. Safety assessments by visit are listed in Appendix 1.

8.1 Targeted Safety Surveillance Items

This study represents the second evaluation of the concomitant administration of leucine, sildenafil and metformin in combination and the initial evaluation of the concomitant administration of leucine and sildenafil in combination. Given that leucine is a naturally occurring amino acid that is regularly ingested as part of normal dietary practices, the well-established safety profiles of sildenafil and metformin when administered at higher doses and the observed safety of the drug combination in preclinical studies to date, the assessed risk is low. However, the fact that this represents the second systematic administration of the leucine, sildenafil and metformin combination and the initial systemic administration of the leucine and sildenafil combination to humans, constant attention to safety monitoring is warranted. The following issues need careful attention throughout the course of the study.

8.1.1 Metformin Associated Lactic Acidosis

Although its occurrence is extremely rare, lactic acidosis has been reported in patients using full dose metformin (1500 to 2500 mg/day). Given that lactic acidosis is a serious condition and the possibility that the leucine-metformin-sildenafil combination might potentiate this effect, study staff need to have a heightened awareness of this possibility.

The initial signs and symptoms associated with lactic acidosis are subtle, vague and non-specific including anorexia, nausea, vomiting, abdominal pain, lethargy and, with advancing severity, hyperventilation and hypotension. Since plasma lactate levels are erratic and of little help until the severity of the disorder has advanced, it is important to be attentive to the electrolyte measurements on the chemistry panel and investigate further any anion gap that is noted. Also, study staff should be attentive to patient reports and implement appropriate clinical evaluation of persistent complaints of lethargy and fatigue. If in doubt, contact the medical monitor. Study medication should be stopped immediately if any evidence of lactic acidiosis is seen.

Also, since intravascular volume depletion can increase the risk for lactic acidosis, the study medication should be discontinued prior to any procedures, surgical or otherwise, that require restricted fluid intake.

8.1.2 Nephrotoxic Effects of Radiocontrast Agents in Patients Taking Metformin

The administration of iodinated radiocontrast agents has been associated with acute nephrotoxic effects when administered to patients taking metformin. Thus, it is recommended that study medication be discontinued for 24 hours prior to the patient receiving any iodinated radiocontrast material. Study medication should then be resumed once the patient has returned to their usual diet and fluid intake.

8.1.3 Risk of Hypoglycemia in Patients Taking Metformin

While metformin is a glucose lowering agent, it is unusual for patients taking **only** metformin to experience hypoglycemia. (Hypoglycemia associated with metformin use is seen almost exclusively when used in combination with a sulfonylurea or insulin.) However, since the experience is limited with leucine, metformin and sildenafil administered together, it is possible that the risk of hypoglycemia could be observed. Therefore, patients will be instructed regarding the signs and symptoms of hypoglycemia and advised to ingest carbohydrate should they encounter the symptoms of hypoglycemia as well as to notify the study site. Study medication should be stopped immediately if any evidence of hypoglycemia is seen.

8.1.4 Hypotension

Sildenafil, at doses as low as 5mg TID, has been shown to cause hypotension. Although rare, if any signs of hypotension are present , in addition to reporting as an adverse event, please notify the medical monitor within 24 hours. Study medication should be stopped immediately if any evidence of hypotension is seen.

8.1.5 Changes in Vision

Sildenafil, at doses of 50 to 100 mg, has been associated with sudden loss of vision. In addition, patients with diabetes mellitus are at increased risk for developing macular edema. While it is unlikely that macular edema will be an

issue in this study of only 24-weeks duration. Should a patient report blurry vision and/or a change in color vision, they should be referred for evaluation to an ophthalmologist.

Sildenafil, at doses of 50 to 100mg, has been associated with non-arteritic anterior ischemic optic neuropathy. Although rare, if any signs of non-arteritic anterior ischemic optic neuropathy are present , in addition to reporting as an adverse event, please notify the medical monitor within 24 hours. Study medication should be stopped immediately if any evidence of non-arteritic anterior ischemic optic neuropathy is seen.

8.1.6 Leucine Toxicity

Leucine, when consumed as a dietary supplement at high doses (>500 mg/kg/day), has been associated with an increase in blood ammonia levels. While it's unlikely that the doses employed in this trial are adequate to elevate blood ammonia levels, the potential for synergism between leucine, metformin and sildenafil exists. Also, the potential exists for the combination to magnify the hypoglycemic effects of metformin.

Therefore, any study participant presenting with neurological symptoms should be evaluated for low blood glucose values and elevated ammonia levels. If either are found, in addition to reporting as an adverse event, please notify the medical monitor within 24 hours. Study medication should be stopped immediately if any evidence of leucine toxicity is seen.

8.2 Reporting of Adverse Events

Adverse events will be reported in accordance with the National Cancer Institute- Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. **(Appendix 2).** The CTCAE grades the severity of the AE based upon Grades 1 through 5 and lists unique clinical descriptions of severity for each AE.

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local or noninvasive intervention indicated or limiting age-appropriate instrumental ADL.

Grade 3: Severe or medically significant but not immediately life-threatening hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL

Grade 4: Life threatening consequences; urgent intervention indicated

Grade 5: Death related to AE

In the event of the occurrence of a same category of three or more grade III CTCAE or 2-grade IV CTCAE or one grade V CTCAE, the trial will be paused, evaluated for safety by the Medical Monitor and allowed to proceed only after the Medical Monitor determines the causality is not associated with the investigational fixed-drug combination.

8.3 Adverse Events and Subject Withdrawals

Adverse Event

Any untoward medical occurrence associated with the use of a therapy in humans, whether or not considered treatment related.

Life-Threatening

Any untoward medical occurrence that places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious Adverse Event (SAE)

ANY SAE THAT OCCURS AFTER THE SIGNING OF THE ICF THROUGH 30 DAYS AFTER ADMINISTRATION OF THE LAST DOSE OF STUDY MEDICATION MUST BE REPORTED IMMEDIATELY (WITHIN 24 HOURS OF KNOWLEDGE) TO THE SPONSOR'S MEDICAL MONITOR (858-342-7057). FAX THE SAE REPORT FORM TO THE NUMBER LISTED ON THE FORM

An adverse event is considered "Serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Any SAE, unexpected events or death encountered during the course of the investigation and for a reasonable time thereafter will be reported to the principal and/or co-investigators by the subject (immediately by telephone and subsequently in writing within five (5) days). The investigator will immediately report the SAE/unexpected event or death within 24 hours of being notified to the sponsor for review and assessment/causality confirmation, and to the IRB (per FDA requirements).

Unexpected Adverse Event:

An adverse event is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed.

FDA guidelines recognize that:

1. "Individual adverse event reports generally require an evaluation of their relevance and

significance to the study, including an evaluation of other adverse events, before they can be considered to be an unanticipated problem," and

2. "All reports to the IRB of unanticipated problems should explain clearly why the event described represents a 'problem' for the study and why it is 'unanticipated.''

Investigators are required to report unexpected adverse events that fit the following criteria *within 10 working days* of the time the investigator has become informed of the event(s) to the IRB and sponsor.

The following guidelines provide further definition to unexpected adverse events:

- Event is **unexpected** (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document, or the investigator brochure; and (b) the characteristics of the subject population being studied,
- **Related or possibly related** to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research **places subjects or others at a greater risk of harm** (including physical, psychological, economic, or social harm) than was previously known or recognized.

If the adverse event is clearly not related to the study drug, device, procedures, or washout process, it would not represent a risk to other subjects in the research or a "problem" for the study and, therefore, does not have to be reported to the IRB.

Intensity:

The intensity of each adverse event will be characterized as mild, moderate, or severe, as follows:

- MILD: Usually transient, requires no special treatment, and does not interfere with the subject's daily activities.
- MODERATE: Usually causes a low level of inconvenience or concern to the subject and may interfere with daily activities, but is usually ameliorated by simple therapeutic measures.
- SEVERE: Interrupts a subject's usual daily activities and generally requires systemic drug therapy or other treatment.

Causality:

The investigator will grade the association of the adverse event as UNRELATED or RELATED to study medication. The following criteria should be considered for determining relatedness:

- UNRELATED: The adverse event is judged to be produced by the subject's clinical state or by other therapies administered to the subject.
- RELATED: The adverse event is judged to be related to the administration of study medication.

Pregnancy:

If a patient becomes pregnant during the study or within 30 days of discontinuing study drug, the Investigator should report the pregnancy to the NuSirt Medical Monitor within 24 hours of being notified. The NuSirt Medical Monitor will then forward the Exposure In Utero form to the Investigator for completion. A patient becoming pregnant while on study drug will immediately be withdrawn from the study and Early Termination study procedures will be performed. A patient who becomes pregnant should be followed by the Investigator until completion of the pregnancy. If the pregnancy ends for any reason before the anticipated date, the Investigator should notify NuSirt Medical Monitor. At the completion of the pregnancy, the Investigator should document the outcome of the pregnancy. If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (ie, postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly) the Investigator should follow the procedures for reporting an SAE. If a pregnancy occurs during the study, the data is to be gathered regardless of the conclusion of the study and provided to NuSirt immediately after collection.

8.4 Clinical Laboratory Evaluations

The investigator or medically qualified physician listed on the form FDA 1572 will evaluate all screening and safety laboratory reports, and will sign and date the review. Any out of range laboratory results should be assessed for clinical significance. The investigator should follow all clinically significant laboratory abnormalities occurring during the study that were not present at baseline. These abnormalities should be evaluated with additional tests, if necessary, until the underlying cause is diagnosed or resolution occurs. The diagnosis and resolution date must be reported to the sponsor. Samples will be collected according to the schedules presented in **Appendix 1** and **Section 6**.

The centralized laboratory will provide specific instructions for collection, processing, packaging, and shipping of all samples.

8.4.1 Chemistry

Chemistry assessments will be performed from blood samples to be collected at Screening/Visit 1 (Day-7/Week-1), Baseline/Visit 2 (Day1), Visit 5 (Day 84/Week 12) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination.

Chemistry assessments will include the following: glucose, urea nitrogen, creatinine, total protein, albumin, uric acid, total and fractionated bilirubin, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltranspeptidase (GGT), creatine phosphokinase (CPK), creatine kinase-MB (CK-MB, If CPK is elevated), sodium, potassium, chloride, bicarbonate, phosphate, calcium, magnesium (or other routine chemistry panels as approved by the sponsor).

8.4.2 Hematology

Hematology assessments will be performed from blood samples to be collected at Screening/Visit 1 (Day-7/Week-1), Baseline/Visit 2 (Day1), Visit 5 (Day 84/Week 12)

and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination

Hematology assessments will include the following: red cell count, hemoglobin, hematocrit, white cell count, platelets, differential count, mean cell volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration (or other routine hematology assessments as approved by the sponsor).

8.4.3 Lipids

Lipid assessments will be performed from blood samples to be collected at Screening/Visit (Day-7/Week-1), Baseline/Visit 2 (Day1), Visit 5 (Day 84/Week 12) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination.

Lipid assessments will include the following: Total cholesterol, HDL, LDL and triglycerides

8.4.4 Urinalysis

Urinalysis assessments will be performed from samples to be collected at Screening/Visit 1 (Day-7/Week-1), Baseline/Visit 2 (Day 1), and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination.

Urinalysis assessments will include the following: pH, specific gravity, glucose, blood, ketones, protein, and microscopic analysis (or other routine urinalysis as approved by the sponsor).

8.5 Other Clinical Laboratory Evaluations

8.5.1 Pregnancy Testing

For all female subjects, regardless of childbearing status (unless subject has had a hysterectomy), pregnancy tests will be performed from blood samples collected at Screening/Visit 1 (Day-7/Week-1).

8.5.2 Urine Drug Screen

A urine screen for drugs of abuse and alcohol will be performed from a urine sample collected at Screening/Visit 1 (Day-7/Week-1). Positive results due to prescription medications (e.g., opioid-containing pain medications) are not necessarily exclusionary as long as it can be documented that they have a prescription.

8.6 Vital Signs, Physical Examination Findings, and ECGs

Clinically significant abnormalities in vital signs, physical examination findings, and ECGs at Study Termination must be followed up by the investigator and evaluated with additional tests if necessary, until the underlying cause is diagnosed or resolution occurs. The diagnosis and resolution date must be reported to the sponsor.

Measurements will be performed as discussed in this section and according to the

schedules presented in **Appendix 1** and **Section 6**.

8.6.1 Vital Signs

Vital sign measurements (heart rate and blood pressure) will be performed for each subject at Screening/Visit (Day-7/Week-1), Baseline/Visit 2 (Day1), Visit 3 (Day 28/Week4), Visit 4 (Day 56/Week 8), Visit 5 (Day 84/Week 12), Visit 6 (Day 112/Week 16), Visit 7 (Day 140/Week 20) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination, according to the schedule presented in **Appendix 1**.

8.6.2 Physical Examinations

A complete physical examination will be performed at Screening/Visit 1 (Day-7/Week-1), Visit 5 (Day 84/Week 12) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination. Complete physical examinations will include evaluation of all body systems listed in the eCRFs.

8.6.3 Electrocardiograms

Standard 12-Lead ECGs will be performed at Screening/Visit 1 (Day-7/Week-1) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination.

Standard 12-lead ECGs will be performed after at minimum 5 minutes of quiet rest with the subject in a supine position. If the ECG must be performed with the subject in another position (sitting, standing, etc.), the investigator should record the alternate position on the ECG eCRF page. This data also needs to be in the medical record. The investigator should date and sign the ECG tracing and record the clinical significance of any abnormal result on the tracing. ECGs will be interpreted by a qualified physician (the investigator or qualified designee) at the clinical study site.

9 BLOOD VOLUME

During this study, blood will be drawn for various analytes and panels, including chemistry, lipids, hematology, and efficacy assessments. The total amount of blood to be drawn (during the entire study) is expected to be <90mL or ~3 ounces for each subject.

10 DISCONTINUING AND UNBLINDING OF SUBJECTS

Every effort should be made to conduct all protocol-required procedures to complete the study. Subjects may be removed from the study for the following reasons:

- Withdrawal of Consent - Subject wishes to withdraw from the study as outlined in the informed consent. All subjects reserved the right to withdraw from the study without prejudice to themselves or their future medical care.
- Adverse Event - Subject experiences an adverse event which, in the investigator's opinion, necessitates withdrawal from the study.
- Investigator Decision - Investigator assessment that it is in the subject's best interest is to terminate participation.

- Protocol Violation – Includes subject non-compliance, documented pregnancy, study entry violation, and/or start of an unacceptable concomitant medication.
- Lost To Follow-Up - Subject fails to return for study visits and cannot be reached with reasonable attempts.
- Administrative Reason – Includes discontinuation of the study protocol by the sponsor, the Food and Drug Administration (FDA), or other regulatory authority, and/or discontinuation of the study site' participation in the study protocol.
- Pregnancy

Any withdrawal from the study will be fully documented on the subject's eCRF and in the subject's study record at the site. The documentation will include reasons for the withdrawal and details of any sequelae (followed through until the symptoms resolved or returned to baseline levels) if the reason was an adverse event.

Every effort will be made to conduct all protocol specified procedures and examinations required to complete the study. Optimally, all procedures should be performed within 24 to 48 hours of termination and, if possible, prior to initiating new therapies. Diligent attempts will be made to follow through endpoint assessment those patients who do not withdraw consent and/or are not lost to follow up.

Only withdrawls for withdrawal of consent or lost to follow-up will not continue to be followed. For all other patients – regardless of whether or not study drug is discontinued – every effort should be made to continue all visits in the study for safety and should be encouraged to complete all study procedures.Subjects who discontinue from the study after randomization and prior to completion of Study Termination /Visit 8 will be considered early withdrawals and, if enrollment is still open, enrollment may be expanded to ensure a full complement of evaluable subjects.

10.1 Unblinding of Subjects

The subject, investigator (including all study-site personnel), and sponsor will be blinded to the identity of the assigned study medication during the 24-week treatment period (Visit 2 to Visit 8). Every effort should be made to ensure that subjects remain blinded to their treatment. The investigator is to notify the sponsor's medical monitor if an event occurs that requires a subject's treatment assignment to be unblinded. The code may be unblinded only after an irrevocable decision has been made to withdraw a subject from the study and if immediate knowledge of the study medication is needed to optimize the clinical management of the subject.

11 DATA MANAGEMENT

A designated contract research organization (CRO) will perform all data management activities, including the writing of a data management plan, outlining the data management system and procedures to be used. The data management plan will detail the procedure for handling and documenting protocol deviations.

Clinical study data will be recorded (captured) electronically by study site personnel on eCRFs. The eCRF will be a validated clinical data management system according to procedures that comply with the Code of Federal Regulations 21 Part 11 and the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (Topic E6, April 2000) Section 5.5.3. The eCRF data will be entered by study-site personnel and then reviewed and electronically signed by the investigator listed on form FDA 1572. All study-site personnel must use an electronic signature access method to enter, review, or correct study data. Electronic signature procedures shall comply with the CFR title 21 part II and the ICH guidelines for good clinical practice (GCP) (Topic E6, April 2000) section 5.5.3. Passwords and electronic signatures will be strictly confidential.

All eCRF data will be downloaded from the electronic data capture (EDC) system and reformatted into SAS data sets. The sponsor's data management department will receive electronic transfers of laboratory data from a central laboratory as well as other data from third-party vendors as appropriate. The electronic data format of all transfers will be agreed upon with the sponsor.

The clinical monitoring staff will verify data recorded in the EDC system with source documents at the clinical study sites according to the data management plan and clinical monitoring plan. The data will be subjected to consistency and validation checks within the EDC system with supplemental validation following download to a SAS data set.

Adverse events will be coded using a current version of medical dictionary for regulatory activities (MedDRA), and concomitant medications using a current version of the WHO drug dictionary. The sponsor will perform a medical safety review of the coding. Completed eCRF images with a date- and time-stamped electronic audit trail indicating the user, the data entered, and any reason for change (if applicable) will be archived at the investigator's site and archived with backup at the sponsor's site.

12 STATISTICAL CONSIDERATIONS

A detailed statistical analysis plan (SAP) describing each of the planned analyses in detail will be prepared and signed before any of the data are unblinded.

12.1 Analysis Populations:

Enrolled: The Enrolled Population will consist of all subjects who signed the informed consent to participate in the study, and had Screening visit electronic case report form (eCRF) collected.

Randomized: The Randomized Populations will consist of all Enrolled subjects who were randomized at Baseline/Visit 2 (Day 1)

Intent-To-Treat (ITT): The ITT Population will include all randomized subjects who receive at least one dose of study medication.

Per-Protocol (PP): The PP Population will include all ITT subjects who had adequate exposure to the randomized study medication (>85%) during the 24-week period, completed Visit 8 (Day 112/Week16) and have adequately complied with the protocol as assessed by the investigator and sponsor prior to database lock. The final definition of the PP Population will be documented in the prospectively developed SAP prior to undertaking any data analysis.

12.2 Study Subjects

Demographic and baseline characteristics will be summarized descriptively by treatment group. Demographic and baseline characteristics include, but are not limited to: gender, age, race, ethnicity, body weight, height, body mass index (BMI), HbA1c, and fasting glucose.

Baseline is defined as the last non-missing observation obtained prior to the administration of the first dose of randomized study medication at Baseline/Visit 2 (Day 1).

12.3 Primary Endpoint Analysis

The primary efficacy endpoint is percentage body weight change from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24).

Descriptive statistics (n, mean, SD, SE, median, min, and max) will be used to summarize values of the primary efficacy endpoint, percentage body weight change from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24) and intermediate study visits as applicable by treatment group. These summaries will be presented for the PP Population using only observed data and the ITT Population employing the LOCF approach.

A mixed-model of analysis of variance (ANCOVA) will be used to analyze change in body weight over the study duration in PP and ITT Populations. The ANCOVA will include treatment group and time as the main effects with baseline weight as the covariate. The LS means, SEs, and the corresponding 95% confidence intervals (CIs) for the changes from baseline to Week 24 will be derived from the model for each treatment. Each of the fixed-dose combinations of leucine, sildenafil, and/or metformin treatment groups (Treatment B, C, D and E) will be compared to placebo group (Treatment A), and the LS means for the treatment difference (Treatment B, C D or E minus Treatment A), the SEs, the associated 95% CIs, and the p-values will be computed.

12.4 Secondary Endpoint Analysis

All secondary efficacy endpoints will be analyzed with mixed-effects models similar to the primary endpoint analyses. For endpoints that require logarithm-transformation to ensure data normality, the change from baseline will be calculated as the log-transformed post-baseline data minus the log-transformed baseline data. These changes from baseline data will be examined as the dependent variable and the log of the baseline covariate will be included as a covariate. The LS means, SEs, and the corresponding 95% CIs for the changes from

Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24) will be derived from the ANCOVA model for each treatment. Each of the fixed-dose combinations of leucine and sildenafil and leucine, sildenafil and metformin treatment groups (Treatment B, C, D and E) will be compared to placebo group (Treatment A), and the LS means for the treatment difference (Treatment B, C, D and E minus Treatment A), the SEs, and the associated 95% CIs and p-values will be computed. The resulting estimates will be exponentially transformed back to the original scale to yield geometric LS mean ratios, SEs of the geometric mean ratios (calculated as geometric LS mean ratio multiplied by the SE of the LS mean of the log-transformed data), and the 95% CIs of the geometric LS mean ratios.

12.5 Safety Analyses

All reported terms (investigator descriptions) for AEs will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA). TEAEs are defined as adverse events with an onset or worsening after the first randomized dose. Number and percentage of subjects with TEAEs will be summarized by treatment group, system organ classification, and preferred term. AEs occurring prior to receiving the first randomized dose (e.g., during the Screening and Pre-Treatment Periods) will not be summarized but will be provided in listings.

TEAEs leading to discontinuation from the study, TEAEs possibly or probably related to study treatment, serious TEAEs, death, TEAEs by severity (or intensity), and TEAEs with maximum severity (or intensity) and causality, will be summarized.

Additionally, TEAEs of special interest, e.g., specific gastrointestinal symptoms, will be summarized separately if deemed necessary.

All hematology, clinical chemistry, and urinalysis results will be listed by treatment group, subject, and visit, including scheduled and unscheduled/repeat measurements. Laboratory assessments that are outside of normal ranges and/or with potential clinical importance will be flagged. Baseline values, the values at each visit, and changes from baseline values will be summarized descriptively for each of the quantitative laboratory assessments by treatment group. Additionally, shift tables of hematology, clinical chemistry, and urinalysis results will be generated to summarize the normal and abnormal (abnormal high and abnormal low) status changes from baseline to each post-baseline visit.

For vital signs, baseline values, the values at each visit, and changes from baseline values will be summarized by treatment group.

ECG and physical examination data will be provided in listing.

13. SAMPLE SIZE AND POWER CALCULATION

A sufficient number of individuals will be screened to enroll at least 250 subjects (50/per treatment). With an assumption of 20% dropout rate, approximately 200 subjects (40/per treatment) are expected to complete treatment through Study Termination/Visit 8 (Day 168/Week 24).

Using a two-sided, two-sample comparison (t-test) of means at the alpha=0.05 level of significance, sample sizes of 23 subjects per treatment will provide approximately 90% power to detect a mean treatment difference of 3 kg body weight (~3.0-3.3% weight loss over 24 weeks), assuming a common standard deviation of 25%. However, detection of significant changes in obesity co-morbidities require a higher sample size; detection of a 6 mm Hg change in blood pressure will require a sample size of 40 subjects per treatment group at 80% power; accordingly, the sample size has been increased to 40/treatment arm to enable assessment of this secondary endpoint. These assumptions of effect size and standard deviation are based on data derived from the previous clinical trial of NS-0200 (NS-0200-01) and represents the anticipated treatment effect of effective fixed-dose combinations of leucine and sildenafil, with or without metformin, compared to placebo

14 INVESTIGATOR AND SPONSOR OBLIGATIONS

14.1 Medical Supervision

Medical supervision is the responsibility of the investigator named on form FDA 1572. The investigator may delegate day-to-day activities to a sub-investigator listed on form FDA 1572, but retains overall responsibility for ensuring that the study is conducted properly and in accordance with the study protocol. A list will be maintained that includes all qualified persons to whom the investigator may delegate significant study related duties. The investigator is responsible for ensuring that drugs and devices are available for treating possible medical emergencies, or that emergency medical facilities are available and accessible. The investigator is responsible for ensuring that the study is conducted according to GCP, other applicable regulatory guidelines, and sound medical practices.

14.2 Study Initiation and Discontinuation

Prior to initiation of the study, the investigator must provide the sponsor with the following documents (copies of which must be retained by the investigator):

- Signed original copy of the protocol acceptance statement (**Section 19**) that commits the investigator to follow the protocol exactly and to conduct the study according to GCP.
- Original copy of the investigator's brochure acceptance page signed by the investigator.
- Completed and signed original form FDA 1572.
- Current curriculum vitae, as indicated by version date in the footer along with the investigator's signature and date. Also required is a state license for the investigator and for other medically qualified sub-investigators.
- Signed financial disclosure forms for the investigator and all sub-investigators listed on form FDA 1572.
- Signed copy of the IRB approval letter that lists the approved items.
- List of the IRB members who voted on the approval, including their specialty and affiliation, or the IRB assurance numbers if the roster cannot be obtained. Copy of

the IRB approved ICF.

- Copy of the IRB approved authorization to use and disclose protected health information form, consistent with HIPAA legislation, if applicable.

Upon receipt of all necessary paperwork, the sponsor will arrange for all study materials to be delivered to the clinical study site. The sponsor or designee will train all personnel expected to be involved in the study. This training may include a review of the study protocol, instructions for eCRF completion, and a review of overall responsibilities, including drug accountability and study file maintenance.

The sponsor has the right to terminate the study at any time for any of the following reasons:

- Non-adherence of study-site personnel to the protocol or GCP
- Unavailability of the investigator or study-site personnel to the sponsor's monitoring personnel or designee(s)
- Other administrative reasons

Additionally, individual subjects may be excluded from the study if a medical records review indicates protocol violations or if other factors appear to jeopardize the validity of the study.

Throughout the course of the study, the investigator is to make a reasonable effort to maintain the enrollment rate that was agreed upon with the sponsor. The investigator will also make a reasonable effort to enroll appropriate subjects. The sponsor may elect to terminate the study at a given site if the enrollment rate lags or if significant numbers of non-evaluable subjects are enrolled.

14.3 Laboratory Accreditation

A central laboratory will be used for all samples in this study, NuSirt Biopharma will provide copies of the central lab accreditation, licensure and related reference ranges.

14.4 Data Reporting

Data for each subject will be recorded on source documents and the eCRF. A source document and an eCRF must be completed at each study visit for every subject enrolled in the study. When data are complete, the investigator or medically qualified sub-investigator listed on form FDA 1572 will apply his/her electronic signature on the eCRF indicating that he/she has reviewed and approves of the data collected on eCRF.

14.5 Study Monitoring

The investigator will allow qualified sponsor representatives or designee(s) to conduct periodic audits and review of all eCRFs, office, clinical, and laboratory records for each subject at each clinical study site. Reviews of study data will be performed during routine monitoring visits, both during the study and following study completion. These visits are to provide the sponsor with the opportunity to evaluate study progress, verify the accuracy and completeness of eCRF, and ensure that all protocol requirements, applicable US FDA or

country-specific regulations, and investigator obligations are being fulfilled. Finally, any inconsistencies in the study records should be resolved during these visits.

The sponsor may terminate study participation by a clinical study site if study-site personnel do not follow the protocol or GCP. Additionally, individual subjects may be excluded if a medical records review indicates protocol violations or if other factors appear to jeopardize the validity of the study.

14.6 Record Retention

Source documents may include a subject's medical record, hospital charts, clinic charts, procedural records, the principal investigator study files, as well as the results of diagnostic and safety tests.

The PI has the responsibility of maintaining a comprehensive and centralized filing system containing all study-related documentation in accordance with the FDA or local regulations, whichever are more stringent. These files must be available for inspection by the sponsor and regulatory authorities (i.e., FDA) at any time and must consist of at least the following elements: subject files, containing the completed electronic case report forms (eCRFs), supporting source documentation from the medical record including laboratory data and the ICF; regulatory files, containing the protocol with all amendments and investigator signature pages, copies of all other regulatory documentation, and all correspondence between the site and IRB and sponsor; and drug accountability files, including a complete account of the receipt and disposition of the test article.

The investigator must maintain study documents: (a) for a minimum of two years following the date the marketing application (NDA) is approved for the indication for which the drug was investigated, or (b) for a minimum of two years following the release date of the final report, if no marketing application is to be filed by the sponsor, or if the marketing application is not approved for the indication of which the drug was investigated or is discontinued and the FDA has been notified, or (c) for a minimum of 15 years after the completion or discontinuation of the study to be filed in support of registration in the European Union. If the investigator relocates, retires for any reason or withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to the sponsor. The investigator must obtain written permission from the sponsor before disposing any study records.

14.7 Deviation from the Protocol

The investigator is not to deviate from the protocol. In medical emergencies, the investigator will use medical judgment and will remove the subject from immediate hazard. The investigator will immediately notify the sponsor and IRB regarding the nature of the emergency and the course of action taken. The investigator is to notify the sponsor of any inadvertent protocol deviations upon discovery, and is to document the deviations appropriately in the study files or on the eCRFs. The sponsor assumes no responsibility or liability for any unapproved deviations.

Major changes in the protocol initiated by the sponsor will be provided as an amendment and

will be approved by the IRB prior to implementation.

14.8 Quality Assurance

NuSirt Biopharma may conduct a discretionary quality assurance audit of this study. If such an audit occurs, the investigator agrees to allow the auditor direct access to all relevant documents and to allocate his or her time and that of the study-site personnel to the auditor to discuss findings and any relevant issues. In addition, regulatory agencies may conduct a regulatory inspection of this study. If such an inspection occurs, the investigator agrees to notify NuSirt Biopharma upon notification by the regulatory agency. The investigator agrees to allow the inspector direct access to all relevant documents and to allocate his or her time and that of the study-site personnel to the inspector to discuss findings and any relevant issues. The investigator will allow NuSirt Biopharma personnel to be present as an observer during a regulatory inspection, if requested.

15 DISCLOSURE OF DATA AND PUBLICATIONS

Subjects' medical information obtained as a result of this study is considered confidential, and disclosure to third parties other than those noted below is prohibited. Subject to any applicable authorization(s), all reports and communications relating to subjects in this study will identify subjects only by initials and number. Medical information resulting from a subject's participation in this study may be given to the subject's personal physician, other authorized parties, or to the appropriate medical personnel responsible for the subject's welfare.

Data generated in this study will be available for inspection on request by the FDA or other government regulatory agency auditors, the sponsor, the sponsor's medical monitor (or designee) and its corporate partners for the study drug, if any, and their designated representatives, the IRB, and other authorized parties.

If requested, the investigator agrees to furnish the sponsor with complete subject identification in a confidential disclosure for long term follow-up if needed. This disclosure will be treated in accordance with applicable law, with strict adherence to professional standards of confidentiality and will be filed by the sponsor with adequate security and restricted accessibility.

All information concerning the study medication and the sponsor's operations (such as patent applications, formulas, manufacturing processes, basic scientific data, or other information supplied by the sponsor and not previously published) are considered confidential and shall remain the sole property of the sponsor. The investigator agrees to use this information only in conducting this study and to not use it for other purposes without the sponsor's prior written consent.

The information developed in this clinical study will be used by the sponsor in the clinical development of this program and therefore, may be disclosed by the sponsor as required, to authorized parties (including its corporate partners for the study drug, if any, and their designated representatives), other clinical investigators, pharmaceutical companies, the FDA, and other government agencies.

Any information, inventions, discoveries (whether patentable or not), innovations, suggestions, ideas, and reports made or developed by the investigator(s) as a result of conducting this study shall be promptly disclosed to the sponsor and shall be the sole property of the sponsor. The investigator agrees, upon the sponsor's request and at the sponsor's expense, to execute such documents and to take such other actions as the sponsor deems necessary or appropriate to obtain patents in the sponsor's name covering any of the foregoing.

The results of this study may be published under the direction of the sponsor. Results will not be published without the sponsor's prior review and approval.

16 ETHICAL CONSIDERATIONS

The study will be conducted in accordance with the Declaration of Helsinki (1964), including the current Seoul revision (2008), and consistent with good clinical practice and applicable laws and regulations.

The protocol and ICF will be reviewed and approved by a duly constituted IRB before individuals are screened for study entry. The investigator will ensure that all aspects of the IRB review are conducted in accordance with current institutional, local, and national regulations. A letter documenting the IRB approval will be provided to the sponsor prior to initiation of the study. Amendments to the protocol will be subject to the same requirements as the original protocol. The investigator will submit all periodic reports and updates that the IRB may require, including any final closeout reports. The investigator will inform the IRB of any reportable adverse events.

17 INFORMED CONSENT

Each individual will be provided with oral and written information describing the nature, purpose and duration of the study, participation/termination conditions, and risks and benefits. Prior to initiation of any study-related procedures, subjects (and their parent or legal guardian for subjects under 18 years of age) will sign and date the ICF to participate in the study. Subjects will also sign and date an authorization form required under HIPAA, if applicable, that authorizes the use and disclosure of the subject's protected health information. The signed original ICF (or child assent form as applicable) and HIPAA authorization forms will be retained with the study center's records and each subject will receive a copy of each form they have signed.

18 FINAL REPORT

All data, including subject characteristics, methodology, and clinical findings, will be presented in a final clinical/statistical report to be prepared by the sponsor or designee.

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20 INVESTIGATOR'S ACCEPTANCE

Study Title: **A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE EFFECT OF TWO FIXED-DOSE LEUCINE AND SILDENAFIL COMBINATIONS (NS-0300) OR TWO FIXED-DOSE LEUCINE, SILDENAFIL AND METFORMIN COMBINATIONS (NS-0200) VERSUS PLACEBO ON BODY WEIGHT IN OBESITY**

Final Date: December 4, 2017

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol. I agree to its terms and will conduct the study according to Good Clinical Practices.

_____
Investigator Name (Printed)

_____ _____
Investigator Signature Date

Appendix 1: Study Plan (Protocol NS-WM-01)

	Screening	24-Week Treatment Period												
STUDY PROCEDURES	**Visit 1 (Screen)**	**Visit 2 (Baseline)**	**Phone Contact**	**Visit 3**	**Phone Contact**	**Visit 4**	**Phone Contact**	**Visit 5**	**Phone Contact**	**Visit 6**	**Phone Contact**	**Visit 7**	**Phone Contact**	**Visit 8 (Study Termination)**
	Day-7 (± 3 day)	**Day 1**	**Day 14 (± 3 day)**	**Day 28 (± 3 day)**	**Day 42 (± 3 day)**	**Day 56 (± 3 day)**	**Day 70 (± 3 day)**	**Day 84 (± 3 day)**	**Day 98 (± 3 day)**	**Day 112 (± 3 day)**	**Day 126 (± 3 day)**	**Day 140 (± 3 day)**	**Day 154 (± 3 day)**	**Day 168 (± 3 day)**
Informed Consent And HIPAA	X													
Inclusion/Exclusion	X													
Medical History	X													X
Adverse Event	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medication Review	X	X		X		X		X		X		X		X
Vital Signs	X	X		X		X		X		X		X		X
Height	X													
Weight	X	X		X		X		X		X		X		X
Waist Circumference		X		X		X		X		X		X		X
Physical Exam	X													X
EKG	X													X
β-hCG	X													
Chemistry and Hematology	X	X						X						X
Blood Lipids	X	X						X						X
hsC-reactive protein		X						X						X
HbA1c	X	X						X						X
Biomarkers		X						X						X
Drug and Alcohol Screen	X													
Urinalysis	X	X												X
Randomization		X												
First Dose Of Study Medication		X												
Return Unused Study Medication				X		X		X		X		X		X
Assess Medication Compliance			X	X	X	X	X	X	X	X	X	X	X	X
Dispense Study Medication		X		X		X		X		X		X		

Maximum study duration is 28 weeks
All visits must be fasting