Statistical Analysis Plan

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 NS-WM-01: 29 August 2017
Protocol Amendment #1: 27 October 2017
Protocol Amendment #2: 16 November 2017
Protocol Amendment #3: 04 December 2017

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This Statistical Analysis Plan has been reviewed and approved by:

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1 INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to describe the statistical methodology to be used in analyzing data in this Phase II, 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. The SAP outlines the data derivations and statistical programming specifications for the tables, figures and listings.

The analyses described in this SAP are based on the Clinical Study Protocol (CSP) NS-WM-01 Amendment #3 dated 04 December 2017, and describes the variables and populations, anticipated data transformations and other details of the analyses not provided in the CSP.

The SAP will be finalized prior to database lock and describes the statistical analysis as anticipated. If circumstances should arise during the study rendering this analysis inappropriate, or if in the meantime improved methods of analysis should come to light, different analyses may be made. Any protocol amendment or significant change between the planned analysis and this SAP that occurs prior to database lock will be addressed in a SAP amendment. If any significant deviation occurs between the planned analysis and this SAP after database lock, the reasons for such deviations and all alternative or additional statistical analyses to be performed will be described in a SAP Addendum.

2 STUDY OBJECTIVES

2.1 Primary Objectives

The primary objective of the study is 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.

2.2 Secondary Objectives

The secondary objectives of the study are 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:

- 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, calculated 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 HbA1c from 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 INVESTIGATIONAL PLAN

3.1 Study Design

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 the five treatment arms in 1:1:1:1:1 ratio (Table 1).

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

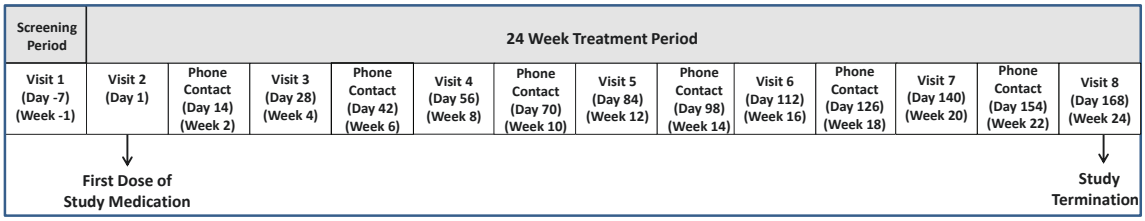
Treatment Arm	Subjects (N) Randomized	Dosing Regimen	Study Medication Dosed Twice/Day[1]		
			Leucine	Sildenafil	Metformin
A	50	Placebo	N/A	N/A	N/A
B	50	FDC	1100 mg	1 mg	N/A
C	50	FDC	1100 mg	4 mg	N/A

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 includes 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 for Protocol NS-WM-01



3.2 Study Procedures and Visit Structure

This study includes a total of 8 visits to the clinical study site 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, Day 98/Week 14, Day 126/ 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). All study visits should occur within a visit window of ±3 days. Total study duration should not exceed a maximum of 28 weeks (or 196 days).

The following study plan (Table 2) provides an overview of the clinical schedule of assessments and events for the study.

Table 2: Study Plan for 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**
	Day-7	**Day 1**	**Day 14**	**Day 28**	**Day 42**	**Day 56**	**Day 70**	**Day 84**	**Day 98**	**Day 112**	**Day 126**	**Day 140**	**Day 154**	**Day 168**
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

3.3 Randomization Schedule and Blinding Procedures

A randomization schedule was provided by Bioclinica's Biostatistician. The randomization schedule was generated utilizing the Bioclinica's Trident® software and is not to be shared with anyone directly involved in the study conduct. Subjects who meet all study requirements based on inclusion and exclusion criteria are to be assigned to one of the five treatment groups on Baseline/Visit 2 (Day 1) (**Table 1**) based on the randomization schedule. The randomization numbers are allocated in strict chronological order. The number uniquely identifies the subject and dictate the assigned, blinded treatment group.

Each randomized subject received a 4-digit randomization number (i.e., 1001, 1002, etc.). Randomized subjects who withdraw or are withdrawn from clinical study participation for any reason, regardless of whether any treatment was administered or not, retain their randomization number and the subject is not allowed to re-enter the study. Replacement subjects are assigned a randomization number as the subject they are replacing plus 1000, i.e., a subject replacing Subject 1001, will be assigned the subject number 2001. The replacement is assigned to the same treatment sequence as the subject being replaced regardless of where the previous subject withdrew.

No stratification factors are planned in this study for randomization or analysis purposes. No cohorts or subgroups are planned in this study for randomization or analysis purposes.

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

3.4 Dose Administration

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

Fixed Dose Combination Arms: Each dose of study medication consists of three tablets - two tablets containing either 550 mg L-leucine alone or 550 mg 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 consists of three tablets, identical in appearance to those used in the active treatment arms – two tablets of leucine alone or leucine + metformin matched placebo and one tablet of sildenafil matched placebo.

3.5 Study Population

A sufficient number of obese subjects will be exposed to study screening 7 days (± 3 days) before the planned start of study dosing. A complete list of all eligibility criteria is available in section 4.2 and 4.3 of the study protocol. The study plans to enroll approximately 250 subjects with obesity, with a maximum of 70% female, with a goal of having at least 200 subjects completing all assessments over the 24-week (up to 28-week) assessment period from multiple study sites in the US. The definition of obesity used for this study is a Body Mass Index (BMI) ≥ 30 kg/m^2. Individuals with a BMI > 45 kg/m^2 will not be included in this study.

4 STUDY ENDPOINTS

4.1 Primary Study Endpoints

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.

4.2 Secondary Study Endpoints

The secondary endpoints of the study are 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:

- The change in absolute body weight from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- The percentage of patients with ≥5% body weight loss from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- The change in waist circumference from Baseline/Visit 2 (Day 1) to Study

Termination/Visit 8 (Day 168/Week 24)

- The change in blood lipids (cholesterol, calculated LDL, HDL, triglycerides) from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- The change in fasting glucose from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- The change in HbA1c from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- The change in blood pressure (diastolic and systolic) from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)
- The change in hsCRP from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)

4.3 Safety and Tolerability Endpoints

Safety and tolerability will be assessed by evaluating the following endpoints:

- Extent of exposure
- Incidence of adverse events
- Adverse events reported by ≥ 5% of patients
- Incidence of deaths and serious adverse events
- Laboratory safety tests (hematology, biochemistry, and urinalysis)
- Vital signs
- ECG
- Physical examination, including weight
- Concomitant medication

4.4 Exploratory Endpoints

- The percentage of patients with ≥10% body weight loss from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24)

In addition, exploratory analysis of comorbidities independent of weight loss i.e. to determine if the comorbidities change occurs independent of the weight loss is also planned.

The comorbidities that will be analyzed are:

- Hypertension i.e. Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP)

- Hyperlipidemia i.e. total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides
- Diabetes Mellitus i.e. fasting blood sugar and HbA1c
- Cardiovascular Diseases i.e. hsCRP

5 ANALYSIS POPULATIONS

All safety analyses will be performed by the actual treatment received.

5.1 Enrolled Population

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. Screen failure subjects will be excluded from the Enrolled Population.

5.2 Randomized Population

The Randomized Population will consist of all Enrolled subjects who were randomized at Baseline/Visit 2 (Day 1) to any of the five study treatments according to the study randomization schedule regardless of whether they receive study drug.

5.3 Intent-to-Treat (ITT) Population

The ITT Population will include all randomized subjects who receive at least one dose of study medication and have at least one follow-up primary efficacy assessment done. It will be assumed all patients who took one dose of study drug will have been assessed for one or more components of efficacy during follow-up, which begins immediately at administration of the first dose. To be included in the ITT population, the date of first dose of study drug must be entered in the eCRF data.

5.4 Per-Protocol (PP) Population

The PP Population will include all ITT subjects who had adequate exposure to the randomized study medication during the 24-week period, completed Visit 8 (Day 168/Week 24) and have adequately complied with the protocol as assessed by the investigator and sponsor prior to database lock. Specifically, subjects should have completed the Visit 8

protocol procedure with valid measurements at Visit 8 (i.e. Week 24 up to Week 28) without major protocol deviations, and have received more than 80% of planned study medication during the treatment period.

PP Population will be the primary analysis population. A memo-to-file will be created if it is necessary to change this selection based on blinded data review on treatment compliance, availability of Visit 8 measurements, and protocol deviation data prior to database lock.

The primary and secondary efficacy endpoints will also be analyzed using both the ITT as well as the PP populations. The safety endpoints will be analyzed using the ITT population.

6 INTERIM ANALYSIS

There is no formal interim analysis planned for this study.

7 STUDY SUBJECTS

All data from the Enrolled Population will be provided in listings. Summary tables will be provided for treatment arms A to E.

7.1 Subject Disposition

The completion status, date of completion or discontinuation, the reason for discontinuation will be listed for the Enrolled population. Also, the record of informed consent and subject status at end of screening will be listed for the Enrolled population.

The subject disposition summary table of analysis populations for all enrolled patients will be summarized by treatment group and overall.

The number of subjects randomized and the frequency and percentage of subjects completing the study, subjects withdrawing early, and primary reason for withdrawal will be summarized by treatment group and overall.

7.2 Demographic and Baseline Characteristics

The demographics and baseline characteristics summary will be presented in tables by treatment group and overall. Descriptive summary statistics will be calculated for continuous variables including age (years) at entry, height (cm), baseline weight (kg) and body mass index (BMI) (kg/m^2), HbA1c, and fasting plasma glucose at screening. This

summarization will be presented by treatment group and overall for ITT Population and the PP Population.

Frequency counts will be tabulated for categorical variables including gender, race, ethnicity and childbearing potential. This summarization will be presented by treatment group and overall for the ITT Population and the PP Population.

All ages will be calculated in Express from date of birth (DOB) in years. Baseline BMI will be derived as follows using baseline weight and height:

BMI (Kg/m^2) = weight (kg) at baseline / (height (m))^2

All patient demographic and baseline data will be presented in a listing for the Enrolled Population.

7.3 Medical History

Medical history will be captured at the screening visit and coded using version 19.0 of the Medical Dictionary for Regulatory Activities (MedDRA®). Medical history will be summarized by system organ class and preferred term. The information of onset and end date and time, or ongoing, will also be collected. All Medical History data will be presented in a listing for Enrolled Population.

Medical history will be summarized in a table with descriptive statistics (frequencies and percentages) by medical history code and treatment group for the ITT Population.

7.4 Inclusion/Exclusion Criteria

The inclusion and exclusion criteria are defined in sections 4.2 and 4.3 of the CSP. All enrolled subjects who did not meet an inclusion criterion and all enrolled subjects who met an exclusion criterion will be presented in a listing.

Inclusion and exclusion criteria failures in each study will be summarized separately (frequencies and percentages) for the ITT Population in a table.

7.5 Treatment Compliance

The compliance ratio for each study medication will be calculated as the total number of received doses of that medication within the 24-week treatment period divided by the total number of required doses of the same medication in the same period.

Treatment compliance percentage for a subject will be defined as:

Treatment Compliance Percentage (%) = Treatment Compliance Ratio x 100

Treatment compliance will be summarized by treatment group for the ITT Population.

7.6 Duration of Exposure

Duration of exposure is defined as the total number of days a patient is exposed to study medication. Duration of exposure is calculated as the total number of days from the first dose date (Day 1) to the last dose date as recorded on the eCRF (last dose date minus first dose date, plus 1). Duration of exposure is therefore the duration of the interval during which the patient was exposed, but not necessarily the number of days on which a patient was exposed if the patient missed dosing on at least one day. If the last day of treatment is missing but Visit 8 occurred, Visit 8 will be used for the last day of treatment for purposes of calculating duration of exposure.

Duration of exposure will be summarized by treatment group for the ITT Population.

7.7 Protocol Deviations

A protocol violation is a deviation from the study protocol that may affect the patient's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. Protocol deviations will be collected and/or computed throughout the study. The deviations include, but are not limited to, patients who:

- Do not meet important inclusion/exclusion criteria
- Incorrect drug dispensed (dispensed different study medication than randomized) and administered
- Have substantial use of any disallowed medication
- Have study treatment compliance < 80% for the assigned treatment
- Have early (before -3 days) or late (after +3 days) visits, inadequate/missed efficacy or safety assessment

All protocol deviations will be recorded by the Investigator and will be listed by subject for the Enrolled Population.

Protocol deviations based on the Inclusion/Exclusion criteria at time of enrollment will be summarized by treatment group for the ITT Population. Other protocol deviations occurring during the trial conduct will also be summarized by treatment group for the ITT Population.

The protocol deviations will be reviewed by clinical and statistical personnel prior to database lock and unblinding, and will be used to determine the subject's eligibility for inclusion in the PP Population.

7.8 Concomitant Medications

The WHO DRUG Dictionary will be used to categorize verbatim descriptions of medications into the Anatomic Therapeutic Chemistry (ATC) classification system. Each verbatim name will be classified by anatomical main group (ATC level 1), therapeutic subgroup (ATC level 2), pharmacological subgroup (ATC level 3), chemical subgroup (ATC level 4), and trade name.

The number and percentage of subjects receiving concomitant and new concomitant medications will be summarized by treatment group and ATC classification (ATC level 2 and level 4) for the ITT Population. Concomitant medications include prior concomitant and new concomitant medications, in which the prior concomitant medications refer to the medications that have a start date prior to receiving the first randomized dose and continue to be taken on or after that, and the new concomitant medications refer to the medications that have been used on or after receiving the first randomized dose.

Dosages for certain concomitant medications should be maintained constant during the study (as mentioned in Section 4.2 and 4.3 of the CSP) 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 and will be captured in prior and concomitant medication listings.

8 STATISTICAL METHODS OF ANALYSIS

8.1 General Considerations

In general, efficacy analyses will be performed with the PP and ITT Populations. Safety analyses will be performed with the ITT Population. Safety analyses will be performed by the actual treatment received.

All data collected during the study for Enrolled Population will be included in data listings.

Only those evaluations that were measured after randomization and events that occur or have onset after randomization will be used in analyses of all efficacy and safety endpoints.

8.1.1 Statistical Notation and Presentation

For descriptive statistical summaries, the mean, sample size (n), standard deviation (SD), standard error (SE), median, minimum (min), and maximum (max) will be calculated for continuous variables. For logarithm-transformed data, the geometric mean and standard error of the geometric mean will also be provided.

For categorical variables, frequency and percentage in each category will be provided. When counts data are presented, the percent will be suppressed when the count is zero to draw attention to the non-zero counts. The denominator for all percentages will be the number of patients in each treatment group with data unless otherwise specified. Data will be summarized for baseline, endpoint and by visit (where applicable).

Min and max values will be rounded to the precision of the original value. Means, least squares (LS) means, and medians will be rounded to 1 decimal place greater than the precision of the original value. SDs, SEs, and 95% confidence intervals (CIs) will be rounded to 2 decimal places greater than the precision of the original value. Percentages for summarizing categorical data will be rounded to one decimal place.

P-values will be presented with 4 decimal places and values less than 0.0001 will be presented as <0.0001.

All dates will be displayed in DDMMMYYYY format. Visits will be referred to as shown in the protocol: “Screening (Visit 1)”, “Baseline (Visit 2)”, “Visit 3”, “Visit 4”, “Visit 5”, “Visit 6”, “Visit 7” and “Visit 8”.

8.1.2 Hypothesis Testing Significance Level

All inferential statistical testing will be two-sided and conducted at the 0.05 significance level. For confidence intervals (CIs) a level of confidence of 95% will be used. No adjustment of type I error for multiple comparisons is planned for this early phase study.

8.1.3 Software

All statistical analyses will be performed using Statistical Analysis Software (SAS®) (SAS® Institute Inc., Cary, North Carolina, United States of America [USA]) Version 9.2 or higher. Graphics may be generated using other software packages.

8.1.4 Definition of Study Baseline

Baseline is defined as the last assessment with non-missing values prior to the first dose of study medication. All baseline measurements must have been collected prior to administration of the first dose of study medication. Measurements that are obtained after the first dose of study medication will be considered as post-baseline values. If a given variable is not measured for a given patient prior to the first dose of study medication, then that patient will be considered not to have a baseline value for that variable. If no baseline values exist for lab measurements, then abnormalities that occur post-baseline will be considered as new abnormalities.

8.1.5 Handling of Multiple Observations

A patient may have multiple scheduled or unscheduled visits that are associated with a protocol defined visit (nominal visit). Each unscheduled visit is assigned a nominal scheduled visit number in the clinical database; therefore, no additional computer programming will be done to attribute the unscheduled visit following specific visit windows. Similarly, there will be no additional computer programming to derive/modify the scheduled visit number even if such a visit falls outside of the protocol-defined visit window.

All values, scheduled or unscheduled, will be presented in data listings. However, multiple observations will be pre-processed before summarizing or analyzing the data. If an unscheduled visit occurs in a visit window with an existing scheduled visit, the assessment at the scheduled visit will be used for data summary and analysis. If no scheduled visit assessment exists for a visit window but at least one unscheduled visit assessment is available within that visit window, then the latest unscheduled visit within the visit window will be used for data summary and analysis.

8.1.6 Handling of Missing Data

Subjects who discontinue from the study prior to completing all study procedures through Study Termination (Day 168/Visit 8), but have data collected for at least one post-baseline visit, will have their missing values imputed using the values at the last

scheduled or unscheduled visit in accordance with the Last Observation Carry Forward (LOCF) approach. This LOCF value will be presented as the value of Endpoint Visit in descriptive statistical summary table. Values at baseline will not be carried forward.

Missing or partial start dates for adverse events (AEs) will be imputed with the following algorithms. Note that for AE start date, the imputation rule is to conservatively capture AEs with missing start dates as treatment-emergent adverse events (TEAEs).

- If "day" is the only missing field, impute the "day" as the later one between the first day of the month and the first dose date if their "month" are the same.
- If "day" and "month" are the only missing fields, impute the "day" and "month" as the later one between January 1 of the year and the first dose date if their "year" are the same.
- If "day", "month", and "year" are all missing, to be conservative, the event will be assumed to occur on the same day as the first dose was administered.

Missing or partial start dates for concomitant medications data will also be imputed using the above algorithms. Missing or partial stop dates for concomitant medications data will be imputed using the following algorithms.

- If "day" is the only missing field, impute the "day" as the last day of the month.
- If "day" and "month" are the only missing fields, impute the "day" and "month" as the last day of the year.
- If "day", "month", and "year" are all missing, leave as missing.

Date imputation will only be used for computational purposes; e.g., treatment-emergent status. Actual data values as they appear in the original CRFs will be shown in the data listings.

8.2 Derivation of Change in Endpoint Parameters

8.2.1 Calculation of Change from Baseline

The change from baseline is defined as post-baseline assessment minus baseline assessment. For example, the absolute change in body weight from Baseline/Visit 2 (Day 1) to Study Termination/Visit 8 (Day 168/Week 24) is defined as the body weight at Study Termination/Visit 8 (Day 168/Week 24) minus the body weight at Baseline/Visit 2 (Day 1).

8.2.2 Calculation of the Percent Change

The percentage change from baseline is defined as post-baseline assessment minus baseline assessment, divided by the baseline assessment, and multiplied by 100:

$$Percentage\ change\ from\ baseline = \left(\frac{Post\_Baseline - Baseline}{Baseline}\right) * 100.$$

The primary endpoint of the percent change from Baseline/Visit 2 (Day 1) in body weight is defined as the body weight at Study Termination/Visit 8 (Day 168/Week 24) minus the body weight at Baseline/Visit 2 (Day 1), divided by the Baseline/Visit 2 (Day 1) body weight value, and multiplied by 100:

$$Percentage\ change\ in\ body\ weight = \left(\frac{Weight_{Study\ Termination} - Weight_{Baseline}}{Weight_{Baseline}}\right) * 100.$$

8.3 Analysis of Baseline Differences

All primary and secondary endpoints will be analyzed for any baseline differences among treatment groups for PP and ITT Populations. All continuous outcomes will be analyzed using an analysis of variance (ANOVA) model and Dunnett's Test will be used to compare each of the active Treatments B, C, D and E with Treatment A (placebo). For all Categorical outcomes, Fisher's Exact Test will be used to compare Treatments B, C, D and E with Treatment A.

8.4 Efficacy Analyses

8.4.1 Primary Endpoint Analyses

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

Descriptive statistics (n, mean, SD, SE, median, min, and max) will be used to summarize values of the primary efficacy endpoint, change in 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.

A mixed-model of analysis of covariance (ANCOVA) will be used to analyze percentage change in body weight from Baseline to Week 24 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% 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.

To make the treatment comparison the following SAS code may be used as reference:

```
PROC MIXED data = dataset ;
CLASS treatment time ;
MODEL change = treatment time baseline ;
LSMEAN treatment / pdiff ALPHA=0.05 CL ;
ESTIMATE 'B vs. A' treatment -1 1 0 0 0  / CL;
ESTIMATE 'C vs. A' treatment -1 0 1 0 0  / CL;
ESTIMATE 'D vs. A' treatment -1 0 0 1 0  / CL;
ESTIMATE 'E vs. A' treatment -1 0 0 0 1  / CL;
RUN;
```

Results of these statistical analyses will be summarized in tables.

8.4.2 Secondary Endpoint Analyses

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.

Proportion of subjects with ≥ 5% body weight loss from baseline will be calculated and summarized for PP and ITT Populations. Pearson's Chi Square Test will be used for analysis of proportions. Fisher Exact Test will be used when any expected cell count will

be less than 5. 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 following statistics will be computed: proportion difference (proportion of subjects for Treatment B, C D or E minus Treatment A), the SEs, the associated 95% CIs, and the p-values.

8.4.3 Exploratory Analysis

Proportion of subjects with ≥10% body weight loss from baseline will be calculated and summarized for PP and ITT Populations. Pearson's Chi Square Test will be used for analysis of proportions. Fisher Exact Test will be used when any expected cell count will be less than 5. 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 following statistics will be computed: proportion difference (proportion of subjects for Treatment B, C D or E minus Treatment A), the SEs, the associated 95% CIs, and the p-values.

NuSirt is interested in analysis of comorbidities independent of weight loss i.e. to determine if the comorbidities change occurs independent of the weight loss. Although this analysis is exploratory in nature and may not provide a true test of treatment effect, it is considered important to examine, and thus will be analyzed with all relevant caveats presented.

The comorbidities that will be analyzed (which are also secondary endpoints) are:

- Hypertension i.e. Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP)
- Hyperlipidemia i.e. total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides
- Diabetes Mellitus i.e. fasting blood sugar and HbA1c
- Cardiovascular Diseases i.e. hsCRP

Analysis methods will follow those of the primary efficacy endpoint. Mixed-model of analysis of covariance (ANCOVA) will be used to analyze change in each comorbidity from Baseline to Week 24 in PP and ITT Populations. The ANCOVA will include treatment group and time as the main effects with percentage of weight loss as the covariate. The LS means, SEs, and the corresponding 95% 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.

Subgroup analysis for group of patients that are above certain threshold limits at baseline for each of the comorbidities will also be performed. Below are the baseline value cutoffs that will be used for the subgroup analysis:

- Hypertension: SPB >130 or DBP>80 mm Hg. Note: Analysis for the change in this comorbidity will be conducted using both parameters for all subjects who meet one or both of these criteria.
- Triglycerides: >150 mg/dL
- Cholesterol: >200 mg/dL
- LDL-C: >130 mg/dL
- HDL-C: <50 (men)/<40 (women). Note: Cutoffs are divided by gender, but analysis will be for all subjects with low HDL.
- FBG: >100
- HbA1c: > 5.7%.
- Hs-CRP: > 3 mg/L

NOTE: Table for the exploratory analysis will have the same specifications as the table for the analysis of secondary endpoints. Therefore, the same shells will be used as the reference.

8.5 Safety Analyses

8.5.1 Adverse Events

The safety and tolerability endpoint is defined as the incidence of treatment-emergent adverse events (TEAEs).

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.

Occurrence of adverse events in each of the active treatment groups (B, C, D, E, B and C pooled, D and E pooled) will be compared to the placebo group (Treatment A) using Pearson's Chi Square Test. Fisher Exact Test will be used when any expected cell count will be less than 5. Statistical tests will be performed for all TRAEs; TRAEs by system organ class, intensity and treatment; TRAEs by system organ class, relationship of study medication and treatment; all serious TRAEs; serious TRAEs by system organ class and treatment; all TRAEs leading to study discontinuation; TRAEs leading to study discontinuation by system organ class and treatment; all drug-related TRAEs; all drug-related serious TRAEs; and frequent (≥ 5%) TRAEs by preferred term and treatment. For each statistical test, Relative Risk Ratio (RRR) will be reported and is defined as the ratio of probability of occurrence of an event in Treatment B (or C, D, E, B and C pooled, D and E pooled) to the probability of occurrence of an event in Treatment A. RRR will be reported along with the associated 95% CI, and the p-value. If any need arises for ad hoc analysis (for example, to explore any trend in occurrence of adverse events with the increase of dose, or exposure-adjusted incidence rate to consider potential time effects), the reasons for such additions and method used for additional statistical analyses to be performed will be described in a SAP Addendum.

8.5.2 Clinical Laboratory Evaluations

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.

8.5.3 Vital Signs

The observed data for blood pressure (systolic and diastolic), pulse, and body temperature will be listed by subject, treatment, and time point. Change from baseline for blood pressure (systolic and diastolic) and pulse rate will be derived and listed by subject, treatment, and time point.

For systolic blood pressure, diastolic blood pressure and pulse rate the observed and change from baseline data will be summarized by treatment and time point. Summaries will include tables of descriptive statistics showing the number of observations (n), mean, SD, median, minimum, and maximum value.

8.5.4 Electrocardiograms

The 12-lead ECG parameters of normal, abnormal without clinical significance, and abnormal with clinical significance on Screening/Visit 2 (Day 1) and Study Termination/Visit 8 (Day 168/Week 24) or Early Termination visit, and changes from baseline values will be summarized by treatment group. Summaries will include tables of descriptive statistics showing the number of observations (n), mean, SD, median, minimum, and maximum value.

8.5.5 Physical Examination

Physical examination data will be listed.

9 SAMPLE SIZE AND POWER

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.

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* This section is intended as a guideline only and is not to constrain in any way the final summary tables, figures, or listings that will accompany the case study report. Additional analyses or modifications will be as needed, and the final table of contents will reflect all analyses as they are performed.

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12 ADDENDUM

12.1 Rationale

This addendum is to document the deviations from the study protocol that were observed after the initiation of the study NS-WM-001. This section clearly explains each deviation and describe proposed actions needed to be taken for the remaining duration of the study, and any effect on the statistical analysis.

12.2 Incorrect Weight Measurement

During the IMV's for the study NS-WM-01, it has come to NuSirt's attention that two sites, Site 09/Young and Site 11/Studdard, have done the weight measurement for all their subjects incorrectly. These two sites took the weight measurement in light street cloths, no shoes and empty pockets instead of no street clothes, no shoes and two gowns as instructed by the protocol. This was done for both Screening/Visit 1 and Baseline/Visit 2 by the time it was discovered by the clinical monitors. As NuSirt is interested in the change in weight during the trial for all participants, the two sites were instructed to continue to weigh all subjects enrolled at their sites on light street clothes, no shoes and empty pockets. This was done to limit skewing of the data by switching to the per protocol method after baseline measurements were already done. Due to this fact all subjects enrolled at these two sites with incorrect weight measurement may be excluded from the statistical analysis of this study. The decision to exclude these subjects will be made prior database lock.

12.3 Antibiotic Use

NuSirt has decided to flag subjects that take antibiotics during the last 30 days of the study. These subjects may have changes in weight that are due to infection or other causes for the antibiotic use. Due to this reason all such subjects will be excluded from the statistical analysis of this study.