A First in Human, Single Center, Single Dose, Randomized, Placebo-controlled, Dose-escalating Study to Evaluate Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Subcutaneously Administered ENT-03S for the Treatment of Obesity and Diabetes	
Clinical Trial Protocol	
Name of Product	ENT-03S
Phase	1a
Trial Protocol Number	ENT-03S-22-001
IND Number	155001
Sponsor	Enterin, Inc. 2005 Market St., Suite 3125 Philadelphia, PA 19103
Protocol Chair	Richard Larson, MD, PhD Chief Medical Officer Email: r.larson@enterininc.com Cell: 505-469-2670
Clinical Trial Protocol Version / Date	Version 13.0 5/20/2024

CONFIDENTIALITY STATEMENT

This study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and conducted in adherence to the study Protocol, Good Clinical Practices as defined in Title 21 of the US Code of Federal Regulations Parts 11, 50, 54, 56, and 312, as well as ICH GCP consolidated guidelines (E6) and applicable regulatory requirements.

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PROTOCOL SIGNATURE PAGE

Authored by:

Signature: _____ Date: __20 May 2024_____

Printed Name: Richard Larson, MD, PhD
Telephone Number: (505) 469-2670
Email: r.larson@enterininc.com

Investigator Agreement

I have read this protocol "A First in Human, Single Center, Single Dose, Randomized, Placebo-controlled, Dose-escalating Study to Evaluate Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Subcutaneously Administered ENT-03S for the Treatment of Obesity and Diabetes" and any auxiliary materials related to Study ENT-03S-22-001 and agree to the following:

1. To conduct the study as described in the protocol, protocol amendment, and any auxiliary materials.

2. To protect the rights, safety, and welfare of the participants in the study.

3. To provide oversight to all personnel to whom study activities have been delegated.

4. To maintain control of all investigational products (IP) provided by the Sponsor and to maintain records of the disposition of those products.

5. To conduct the study in accordance with the protocol, all amendments, and all applicable local and national regulations, including, but not limited to, the International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (GCP), and the standards set forth by the ethical principles that have their origin in the Declaration of Helsinki, GCPs as indicated in Food and Drug Administration (FDA), ICH E6 (R2) and ICH E3.

6. To obtain approval for the protocol, protocol amendment, and all written materials provided to participants prior to initiating the study at my site.

7. To obtain informed consent from all participants enrolled at my study site prior to initiating any study-specific procedures or administrating IP to participants.

8. To maintain accurate records of each patient's participation.

_____

Investigator's Signature Date

STUDY SYNOPSIS

Study Title:

A First in Human, Single Center, Single Dose, Randomized, Placebo-controlled, Dose-escalating Study to Evaluate Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Subcutaneously Administered ENT-03S for the Treatment of Obesity and Diabetes

Protocol Number: ENT-03S-22-001

Study Phase: 1a

Methodology:

This study will be conducted as a single-center study in the United States (US). Total study duration will be approximately 12 months. A single dose of ENT-03S, escalating over time, and depending on tolerance, will be administered subcutaneously to each subject with a 14-day follow-up period. Up to 49 subjects will be enrolled. Cohorts will receive either 3 mg (Cohort 1), 6 mg (Cohort 2), 12.5 mg (Cohort 3), 25 mg (Cohort 4), 50 mg (Cohorts 5, 6), or 75 mg (Cohort 7) of ENT-03S using 5:2 (active: placebo) randomization. Sentinel dosing will be used, consisting of enrolling three subjects at a 2:1 active to placebo ratio followed by the remaining subjects in the respective dose cohorts. Each of the 7 dose cohorts will enroll five active and two placebo subjects, with a total of up to 35 subjects receiving active therapy across the 7 arms and 14 subjects receiving placebo. Cohorts 1 through 5 will enroll subjects with obesity who are otherwise healthy. Cohorts 6 and 7 will enroll subjects with obesity and Type 2 diabetes. Cohort 7 will be optionally dosed depending on the results from cohort 6. The study will be conducted on an in-patient basis with outpatient visits at Screening and on Days -7, 3, 4, 5, 6, 7, 8, and 14 (End of Study).

Subjects in cohorts 4, 5, 6, and 7 will have fasting and postprandial blood samples taken for glucose and insulin testing on Days -7, 2, 3, 4, and 8.

Subjects will be advised that additional testing for inflammatory markers may be performed on serum samples obtained during the course of the study at the Sponsor's discretion.

The Investigator or Sponsor will interrupt the scheduled dose escalation scheme in one of the following cases:

- Experience of one severe or multiple moderate adverse events in 50% or more subjects per dose level. This dose is considered the minimal intolerable dose (MID) and the dose below is then considered the maximum tolerable dose (MTD).
- One or more subject(s) of the dose group experience(s) an adverse event, which may jeopardize the subject's health at Investigator's judgment and whose relation to study drug is considered as possibly or probably related.

Number of Patients:

Up to 49 subjects will be enrolled into the study.

Study Duration:

Each subject will participate in the study for approximately 4 weeks (inclusive of the screening period).

Study Centers: This is a single center trial in the US.

Objectives:

Primary Objective:

- To determine the safety and tolerability of a single subcutaneous dose of ENT-03S in obese but otherwise healthy subjects and in subjects with obesity and Type 2 diabetes.

Secondary Objectives:

- To determine the pharmacokinetics of a single subcutaneous dose of ENT-03S.
- To determine the pharmacodynamics of a single subcutaneous dose of ENT-03S in improving fasting blood glucose and insulin, leptin, lipid profile, and body weight. All endpoints will be measured at baseline (Screening Visit), at 72 hours (Day 4), and at 168 hours (Day 8).
- To determine the effect of a single subcutaneous dose of ENT-03S in improving fasting and post-prandial blood glucose and insulin, insulin sensitivity as measured by homeostatic assessment of insulin resistance (HOMA-IR) and Matsuda Index, in subjects with obesity and Type 2 diabetes (cohorts 4, 5, 6, and 7). Endpoints will be measured on Days -7, 2, 3, 4, and 8.

Study Description:

Single center, single-dose, randomized, placebo-controlled, dose-escalating study to evaluate safety, tolerability, pharmacokinetics, and pharmacodynamics of escalating doses of ENT-03S in obese but otherwise healthy subjects and in subjects with obesity and Type 2 diabetes.

Study Population:

Obese but otherwise healthy subjects and obese subjects with Type 2 diabetes (cohorts 6 and 7 only)

Study Drug Administration:

Subcutaneous injection in the abdomen

Inclusion/Exclusion Criteria:

The study population is defined as subjects who meet the following criteria:

Inclusion Criteria:

1. Subjects aged 18-70 years, both genders.
2. Healthy as determined by a physician, based on history, medical examination, vital signs, and laboratory tests.
3. Males that agree to use condoms during the duration of participation in the study.
4. Females of non-child-bearing potential (i.e., tubal ligation, hysterectomy, or

postmenopausal).

5. Female patients of child-bearing potential with negative serum pregnancy tests and who agree to use double barrier contraception during the study.
6. Subjects must be able to read, speak, and understand English and/or Spanish and provide written informed consent, and are willing and able to comply with study procedures.
7. Subjects with a BMI 30-35 kg/m^2 (inclusive), assessed at screening.
8. HbA1c 6.5% -8.5% (cohorts 6 and 7).
9. Subjects with Type 2 diabetes on no anti-diabetic medications or on stable doses of metformin for 4 weeks or more (cohorts 6 and 7).
10. No history of active or chronic disease other than that allowed by study (all cohorts: hypertension, hyperlipidemia, hyperglycemia, GERD, or heartburn; cohorts 6 and 7 only: preceding conditions plus Type 2 diabetes).

Exclusion Criteria:

1. History of excessive alcohol use (defined as ≥21 drinks per week for males and ≥14 drinks per week for females), recreational drug use within the past three months, or failure on urinary drug screen.
2. Pregnant or breastfeeding within six months of screening assessment.
3. Substantial changes in eating habits or exercise routine within the preceding three months.
4. Evidence of eating disorders.
5. ≥5% weight change in the past three months.
6. Bariatric surgery within the past five years.
7. Significant renal impairment (eGFR <60 mg/mL/1.73m^2).
8. Patients on anti-diabetic medications other than metformin.
9. Patients with gastroparesis.
10. Patients with Type 1 diabetes.
11. Liver function tests (i.e., ALT, AST, alkaline phosphatase, bilirubin) exceeding the upper limit of normal by greater than two-fold.
12. Diseases interfering with metabolism and/or ingestive behavior (e.g., myxedema, Cushing's disease, schizophrenia, major psychoses).
13. History of major depressive disorder within the previous two years, a lifetime history of suicide attempt, suicidal behavior within the previous month, or history of other severe psychiatric disorders.
14. Score of ≥15 on the Columbia Suicide Severity Rating Scale (C-SSRS).
15. Use of medications affecting body weight within the past three months:
    - 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

16. Any clinically significant abnormality following the Investigator's review of the physical examination and clinical laboratory tests.
17. A baseline prolongation of QT/QTc interval after repeated measurements of >450 ms; a history of unexplained syncope, cardiac arrest, unexplained cardiac arrhythmias or torsades de pointes, structural heart disease, or a family history of Long QT Syndrome (LQTS). Eligibility should be assessed using the $QTc_{F.}$value.
18. Participation in an investigational drug trial within the month prior to dosing in the present study.

Concomitant Medications:

Beginning on Day -7 and continuing through the end of study visit, all subjects must abstain from regular use of prescribed or OTC drugs, food supplements, and herbal remedies with the exception of the following: antihypertensives, statins, antihistamines, prescription or OTC medications for heartburn and GERD, OTC pain relievers (i.e., acetaminophen, ibuprofen, naproxen), hormonal birth control, and vitamins. Subjects with Type 2 diabetes enrolled in cohorts 6 and 7 may use metformin; however, no other antidiabetic medications are allowed.

Study Product, Dose, Route, Regimen:

Five subjects in cohort 1 will be administered a single subcutaneous dose of 3.0 mg of ENT-03S and two subjects will receive placebo subcutaneously. The cohort will be staggered via sentinel dosing with two active and one placebo patients treated on Day 1 and the remainder dosed on Day 4 if there are no safety concerns. The same dosing regimen will be observed for each incremental cohort/dose level (i.e., 6.0 mg through 75 mg).

The highest dose that is safe and tolerable in all subjects will be determined as the MTD. Blood samples will be obtained for PK analysis before drug administration and at 30 minutes, 60 minutes, 90 minutes, and 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours after administration.

Primary Safety Endpoints:

- AEs
  - Number of Participants with Treatment-emergent Adverse Events (TEAEs)
  - Number of subjects with clinically significant changes in clinical laboratory tests reported as Treatment-emergent Adverse Events (TEAEs) clinical laboratory tests included hematology, chemistry, and urinalysis.
  - AEs of special interest: The frequency of clinical symptoms of nausea, vomiting, and injection site discomfort or irritation. In the event an injection

site reaction occurs, clinical staff will photograph the site reaction for inclusion in the case report file.

- Physical examination (full or abbreviated)
- Vital signs
- Clinical chemistry
- EKG (time matched with PK sampling for QTc analysis)

Secondary Pharmacokinetic Endpoints:

- Area under the curves (AUC) for different time-intervals: i.e., $AUC_{0\text{-}1h}$, $AUC_{0\text{-}2h}$, $AUC_{0\text{-}4h}$, $AUC_{0\text{-}12h}$, $AUC_{0\text{-}24h}$, AUC_{inf}
- Maximum ENT-03S concentrations (C_{max})
- Time to maximum ENT-03S concentrations (T_{max})
- Half-life ($t_{½}$), clearance rate (CL/F), volume of distribution (V/F), and slope of the terminal elimination phase (λ_z)
- Dose-exposure relationship based on C_{max} and $AUC_{0\text{-}12h}$

Secondary Pharmacodynamic Endpoints:

- Change from baseline (Screening Visit) in
  - Fasting plasma glucose
  - Fasting serum insulin
  - Fasting lipids (TC, HDL-C, LDL-C, Triglycerides and FFA)
  - Fasting leptin
  - Body weight
  - Mixed Meal Tolerance Testing for insulin sensitivity by HOMA-IR and Matsuda Index in cohorts 4, 5, 6, 7 on Days -7, 2, 3, 4, and 8

Statistical Methods:

Safety Population: The Safety Population will consist of all patients who receive at least one dose of study medication during the study with patients to be analysed based on the actual study treatment received.

Pharmacokinetic Population: The Pharmacokinetic Population will consist of the Safety Population subjects for whom at least one PK parameter can be derived.

Analysis Methods:

No formal statistical hypotheses will be tested. Descriptive summary statistics will be presented for the safety and tolerability endpoints overall. Continuous variables will be summarized using the number of observations, mean, standard deviation, median, minimum, and maximum. Categorical variables will be summarized using frequency counts and percentages. Summary statistics will be provided by cohort, pooling the placebo subjects as appropriate.

Dose-proportionality for C_{max} and AUC_{inf} will be analyzed using an ANOVA of the natural log-transformed AUC_{inf} and C_{max}.

The site PI and the Sponsor's CMO will review the blinded data after the sentinel group and will have the option of allowing the remaining patients to be randomized to the same dosing group. The site PI and the Sponsor's CMO will also review the blinded results of clinical findings, lab results, and PKs (where available) from each dosing group before escalating to the next dosing group. Additionally, the Sponsor's CMO will review the unblinded results at the end of each cohort.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse Event
CIOMS	Council for International Organizations of Medical Sciences
CFR	Code of Federal Regulations
CRF	Case Report Form
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Tolerability
EMEA	European Agency for the Evaluation of Medicinal Products
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IB	Investigator's Brochure
ICF	Informed Consent Form
IEC	Independent Ethics Committee
IRB	Institutional Review Board
MTD	Maximum Tolerable Dose
SAE	Serious Adverse Event
TEAE	Treatment Emergent Adverse Event
ULN	Upper Limit of Normal
WHO	World Health Organization

1 BACKGROUND

The prevalence of obesity has markedly increased over the past three decades and is now a major public health challenge in the US. Prevalence of obesity, defined as a BMI $\geq$ 30 kg/m^2 for adults, is 37.7% among US adults (1). Prevalence is higher among women than men (40.5 vs. 35.2%), and minority women are disproportionately affected, with a prevalence of 46.6% among Hispanic women and 57.2% among Black women in the US (1). Furthermore, while the trend for men has levelled off in the last decade, it continues to increase among women (1).

Multiple large studies have shown that obesity reduces lifespan (2-6), with a predicted loss of 9-13 years of life for individuals with BMI $\geq$35 (2). The Global BMI Mortality Collaboration recently reported data from a meta-analysis of 10.6 million participants followed an average of 14 years culled from 239 large studies, including 189 studies that included 4 million participants who were never-smokers (6); the data demonstrate a 31% increase in risk of premature death for every 5 BMI unit increase over 25, and an overall increased risk of 45% for stage 1 obesity, 94% for stage 2 obesity, and ~3-fold for stage 3 obesity (6).

Obesity increases the risk of co-morbidities, including cardiovascular disease (CVD), type 2 diabetes (T2D), hypertension, dyslipidemia, obstructive sleep apnea, non-alcoholic fatty liver disease (NAFLD), some cancers and Alzheimer's disease (AD) (7,8). The risk of obesity-related co-morbidities increases with increasing BMI; for example, there is an estimated 27% increase in risk of coronary heart disease (CHD) and 18% increase in risk of stroke for each 5 kg/m^2 elevation in BMI (9). Obesity is not only more common among minority populations such as blacks and Hispanics, but it also has a disproportionate adverse health impact (10, 11), and the outcomes of weight loss trials in these populations are less successful than among white participants (12) Finally, the economic cost of overweight and obesity in the US, (calculated as the sum of excess medical costs, mortality and loss of productivity due to obesity-induced disability) is approximately $300 billion per year; $80 billion of this is attributable to overweight and $220 billion to obesity (13).

The prevalence of T2D has nearly doubled worldwide since 1980, increasing from 4.7% to 8.5% of the adult population (14-16). In the US alone, 37% of the population or 86M adults have pre-diabetes, and 29M people have T2D (14; 17-22). In addition to increasing cardiovascular risk, T2D also dramatically increases the likelihood of developing cognitive impairment, dementia and

AD, a condition referred to by some as “central insulin resistance” or Type 3 Diabetes (T3D) (23-25).

Modest reductions in BMI resulting from as little as 5-10% weight loss significantly reduces the risk and severity of obesity co-morbidities, with continued improvement with greater weight loss (8, 26). Lifestyle intervention focused on reducing caloric intake and increasing caloric expenditure are the first-line approach to obesity management; however, although short-term weight loss frequently occurs, long-term patient adherence is generally poor. Consequently, weight regain following weight loss is common, 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.

There are presently five therapeutic agents approved for weight management, with four approved in the past decade (27, 28). Of these recent approvals, one (Lorcaserin) was withdrawn from the market at FDA request in February 2020 due to elevated cancer risk. Further, for all of these agents, the number of patients needed to treat to achieve sustained weight loss $\geq$5% in a single individual range from 4-5 for the newer agents and 10-12 for Orlistat (28); this indicates that currently available agents do not meet the needs of many obese individuals who could benefit from weight loss. In addition, adverse medication effects likely to prompt discontinuation may affect 20-25% of patients treated with Orlistat, Liraglutide, Semaglutide, and phentermine/topiramate, and 5-7% of those treated with Bupropion/naltrexone (28). Accordingly, while currently available weight management medications are efficacious, they do not fully address the needs of the obese patient population, and there continues to be an unmet need for safe and effective treatment alternatives.

The hypothalamus plays an essential role in monitoring and regulating appetite, body weight and energy homeostasis (29). Hypothalamic peptides such as NPY, AGRP, and PMOC, and hormones such as insulin, leptin, and IGF1 all play a role in the control of body weight and energy balance within hypothalamic neural circuits. These hypothalamic signaling pathways are regulated by protein phosphatases such as PTPN1/PTP1B and PTPN11/SHP2 and are profoundly deranged in conditions ranging from obesity to Type II diabetes and Alzheimer’s disease. Common to these conditions is resistance to the physiological effects of insulin at the level of the brain (30). Efforts to reverse central insulin resistance have focused on pharmacological inhibition of PTP1B based on compelling studies in PTP1B knockout mice (31). Rationally designed PTP1B-specific inhibitors, however, have had

limited success in reversing insulin resistance and reducing body weight in humans due in large part to poor pharmacodynamic characteristics (32,33).

Trodusquemine (MSI-1436), a steroidal compound isolated from the liver of the dogfish shark (34), was discovered to inhibit PTP1B both *in vitro* and *in vivo* (35-37). The mechanism of PTP1B inhibition appears to be allosteric, with two specific binding sites identified (38). Studies *in vivo* demonstrated that trodusquemine inhibited the dephosphorylation of both the insulin receptor and Stat3 (a leptin signaling protein (39)) within the hypothalamus. Trodusquemine acts on hypothalamic centers involved in appetite and energy balance, suppressing the expression of the orexigenic peptides, AGRP and NPY (40-42). When administered to obese mice, trodusquemine reduces food intake, adiposity and body weight (43), corrects insulin resistance and reverses fatty liver disease (40). It reverses atherosclerosis in LDL receptor knockout mice (44); it inhibits the growth of malignancy; stimulates regenerative repair of myocardial infarction by mobilizing stem cells in adult mice (47); reverses memory impairment, normalizes behavior and reduces neuronal loss in a beta amyloid mouse model of Alzheimer's disease via a neuronal PTP1B dependent mechanism (45); and reduces toxic alpha synuclein aggregate formation and increases in lifespan in a *C. elegans* Parkinson's model (46). In early-stage human clinical trials involving subjects with obesity and diabetes, it caused dose-dependent weight reduction and increased insulin sensitivity (48).

1.1 Rationale for the Study

ENT-03S is the mammalian homologue of trodusquemine. It shares an amphipathic chemical structure with trodusquemine, and unsurprisingly, also shares comparable biophysical properties. It is present in neonatal mouse brain, kidney, and GI tract. The highest concentrations are found in brain, peaking at 1 week of age and declining rapidly thereafter. Body weight remains low while ENT-03S levels are high and increases rapidly as ENT-03S levels decline, suggesting that this hormone may have a role in balancing body weight in relation to energy supply.

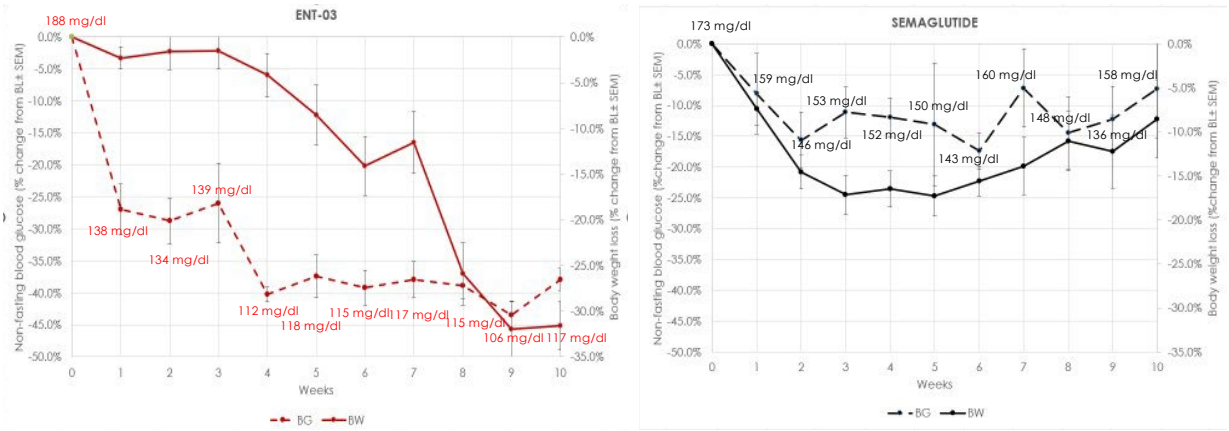
ENT-03S exhibits potent inhibitory activity *in vitro* against several human protein phosphatases, including PTPN1/PTP1B, PTPN2, PTPN6, PTPN7, PTPN11/SHP2 and PTPN12, PTPRC and DUSP22. The most potent inhibitory activity is against PTP1B with an IC50 of 0.117μM, similar in magnitude to trodusquemine (35). Like ENT-03S, trodusquemine also exhibits comparable activity *in vitro* against other protein phosphatases, a property that contrasts starkly with the highly specific compounds being developed through rational design to target a specific signaling pathway.

Peripheral administration of ENT-03S results in a dose-dependent decrease in food intake and body weight. Following intraperitoneal administration of ENT-03S, animals dosed at 3 mg/kg gained weight at about half the rate of untreated animals; at 5 mg/kg, they did not gain weight, and at 10 mg/kg they lost a maximum of about 3%, and about 9% relative to vehicle-treated animals. Although weight gain resumed after the cessation of dosing, animals did not recover body weight over the 2-week post treatment observation period. The effects of ENT-03S on weight are similar to those of trodusquemine (40,42,43).

ENT-03S appears to readjust the body weight set point in rats following intra-cerebral administration. A single intraventricular dose of 60 or 120 µg (about 0.17 mg/kg and 0.34 mg/kg, respectively) of ENT-03S causes an acute decrease in weight over the first 1-2 days after injection. Animals dosed with 120 µg of ENT-03S experienced a maximum weight loss relative to vehicle of about 14%. Food intake decreases during the period of acute weight loss. While the animals continue to increase in weight, they adjust food intake to maintain their reduced body weight relative to untreated rats, suggesting they have established a new body weight set-point.

Peripheral administration of ENT-03S to obese, diabetic mice results in a dose-dependent improvement in blood glucose and insulin. Following a single dose administered intraperitoneally to ob/ob mice, blood glucose and insulin normalized. Additionally, following 5 or 6 doses administered over a 2-week period, liver function tests normalized, and the severity of hepatic steatosis was markedly reduced in the ENT-03 treated animals but much more so than in the pair-fed animals, indicating that the effects are greater than can be explained solely by weight loss and food restriction.

In a diet-induced obesity mouse model (DIO), ENT-03 (25mg/kg), semaglutide (0.04mg/kg) or vehicle were administered subcutaneously twice weekly for 10 weeks (n=5/group). In ENT-03 treated mice on a high fat diet, non-fasting glucose rapidly fell to within normal range prior to any significant weight loss ($p=2x10^{-5}$), remaining normal thereafter. In contrast, in semaglutide treated mice, glucose fell in proportion to weight loss, remaining elevated throughout (p=0.2). ENT-03 treated mice were restored to lean body weight. Body weight and glucose improved partially in semaglutide treated mice. Body fat decreased by 49% in ENT-03 ($p=10^{-5}$) and by 19% in semaglutide treated mice (p=0.1). Basal glucose uptake in muscle was increased 3-fold in ENT-03 treated mice compared to controls.



ENT-03 is a novel endogenous, centrally acting mammalian steroid which rapidly normalizes glucose, independent of body weight and causes gradual but marked weight loss in both genetic and diet-induced models of obesity and diabetes.

C-Fos imaging of the adult mouse brain following systemic administration of ENT-03S highly stimulated multiple hypothalamic areas involved in appetite suppression, food entrained circadian activity and autonomic function, as well as areas of the brainstem involved in visceral autonomic responses, indicating that it acts via a central connectome to regulate body weight and energy homeostasis.

This study is designed as a first-in-human trial to assess safety and tolerability, with secondary pharmacokinetic and pharmacodynamic endpoints.

1.2 ENT-03S Dosing Rationale

The starting dose and dose range for the proposed Phase 1 clinical trials of ENT-03S is based on prior Phase 1 studies of the closely related compound, trodusquemine (MSI-1436), as noted above. ENT-03S was discovered by Enterin as the mammalian homologue of trodusquemine and the two compounds differ structurally only in the replacement of the sulfate ester on C-24 in trodusquemine with a carboxylic acid at C-26 in ENT-03S. The two compounds have comparable affinity for PTP1B, exhibit comparable pharmacokinetics in rodents and comparable pharmacological activity in rodents at the same dose levels. In the first Phase 1 study of trodusquemine (MSI-1436C-101) single doses were well tolerated at doses of 0.3 – 40 mg/m^2 (~0.5 – 70 mg), with nausea and infusion site reaction (grade 1) being the primary AE at the highest dose (70 mg). A second Phase 1 trial (MSI-1436C-103) confirmed these findings, with single doses of

3-15 mg/m^2 (~5 – 25 mg) trodusquemine being well tolerated and safety signals/AE profile comparable to placebo.

7 day and 28 day toxicokinetic studies have been conducted in both rats and dogs. Animals were dosed subcutaneously with the clinical formulation once every 48 hrs. Administration of ENT-03S to both male and female dogs once every other day for 28 days by the subcutaneous injection (up to 14 dose in total) at dosages of 2.28, 5.32 or 10.64 mg/kg/dose followed by a 14-day recovery period was well tolerated and did not result in any treatment-related mortality, and any adverse effects in clinical signs, body weight, body temperature, food consumption, ophthalmology, clinical pathology, safety pharmacology and pathology. Decreased body weight was observed at $\geqslant$ 2.28 mg/kg/dose and decreased food consumption was observed at $\geqslant$ 5.32 mg/kg/dose during the dosing period, but the decrease was reversible following the completion of the 14-day recovery period. Accordingly, the no observed adverse effect level (NOAEL) for this study was considered to be 10.64 mg/kg/dose. By allometric scaling the equivalent human dose would be about 319 mg for a 60 kg person.

Administration of ENT-03S to both male and female Sprague Dawley rats once every other day for 28 days by the subcutaneous injection (up to 14 doses in total) at dosages of 10, 30 or 45 mg/kg/dose followed by a 14-day recovery period was generally well tolerated and did not result in any treatment-related mortality, and any adverse effects in clinical signs, body temperature, ophthalmology, clinical pathology, safety pharmacology and pathology. However, there were deaths in two dose groups that, while believed to be opportunistic sepsis unrelated to ENT-03S, were not fully explained, preventing determination of a NOAEL in rats. Accordingly, a second study was conducted in the dose-range of 5-45 mg/kg. No adverse events were observed at doses up to 30 mg/kg, and the NOAEL for the rat was therefore considered to be 30 mg/kg. Decreased food intake and body weight were observed at all doses, in dose dependent fashion in both studies. The decrease in both food intake and body weight reversed during the 14 day recovery. Allometric scaling of the 30 mg/kg NOAEL for the rat provides a human equivalent dose of approximately 290 mg for a 60 kg person.

Based on these observations, Enterin proposes to study ENT-03S in the first-in-human single ascending dose trial with a starting dose of 3.0 mg, which is more than 100 fold lower than the established NOAEL observed in the toxicokinetic studies. In addition, ENT-03S is an endogenous

mammalian compound and, as such, is likely to be tolerated at least as well as the closely related shark homologue (trodusquemine).

At a dose of 12.5mg (cohort 3), the AUC_{0-48} was 39 times lower than the NOAEL. The proposed top dose to be evaluated in this clinical trial is 75 mg, a dose that is estimated to be 25% of the NOAEL. However, exposure will be monitored following each cohort to ensure that an AUC exposure cap of 414 µg*h/mL (10% of the exposure found in rats at the dose above the NOAEL) will not be exceeded in any subject.

2 OBJECTIVES

The purpose of this study is to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of subcutaneously administered single doses of ENT-03S at doses ranging from 3.0 mg to 75 mg in subjects with obesity and obese subjects with Type 2 diabetes. A second objective of the study is to determine the effect of subcutaneously administered ENT-03S on glycemia, insulin, insulin sensitivity, leptin, lipids, and body weight.

2.1 Primary Objective

The primary objective of the Phase 1a study is to determine the safety and tolerability of a single subcutaneously administered dose of ENT-03S in obese but otherwise healthy subjects and in individuals with obesity and Type 2 diabetes. Each subject will receive a single dose of ENT-03S at doses ranging from 3.0 mg to 75 mg or placebo.

2.2 Safety Assessments

The safety endpoints will be adverse events as measured by patient report, physical examination, vital signs, clinical chemistry, and EKG.

It is anticipated that no more than 1 subject out of 35 (2.9%) receiving ENT-03S will have an adverse event of grade 4 or 5 that is at least possibly related to ENT-03S. Further dosing within a given dose cohort after sentinel dosing, as well as dose escalation to higher dosing cohorts, will be suspended if any subject experiences an SAE/life-threatening event possibly related to ENT-03S. The data will be reviewed by the site PI and the Sponsor's CMO and submitted to the Agency before proceeding. Safety Monitoring processes are identified in Section 6.

2.3 Tolerability Endpoints

Tolerability endpoints (referred to as the dose-limiting tolerability, DLT) will be:

- Severe nausea within 24 hours of taking drug
- Severe injection site reaction within 72 hours (grade ≥3)

If this occurs, antiemetic medication or analgesics will be administered as seen fit by site PI. Injection site discomfort will be treated with topical Benadryl and topical cortisone cream 1%. In the event an injection site reaction occurs, clinical staff will photograph the site reaction for inclusion in the case report file. Appropriate times for assessment are 12, 24, and 72 hours.

The site PI and the Sponsor's CMO will review the blinded data after the sentinel group and will have the option of allowing the remaining patients to be randomized to the same dosing group. At the end of each cohort, the site PI and the Sponsor's CMO will review the blinded results of clinical findings and lab results from each dosing group. The Sponsor's CMO will also review the unblinded data (including PK results), and assuming there are no concerns and that the exposure cap is not projected to be exceeded in the next dose cohort, the Sponsor's CMO will advise the site PI that the next cohort can be treated.

2.4 Secondary Objectives

- To determine the pharmacokinetics of a single subcutaneous dose of ENT-03S.
- To determine the effect of a single subcutaneous dose of ENT-03S in improving fasting blood glucose and insulin, leptin, lipid profile, and body weight. All endpoints will be measured at baseline (Screening), at 72 hours (Day 4), and 168 hours (Day 8).
- To determine the effect of a single subcutaneous dose of ENT-03S in improving fasting and post-prandial blood glucose and insulin, and insulin sensitivity as measured by homeostatic assessment of insulin resistance (HOMA-IR) and Matsuda Index in subjects in cohorts 4, 5, 6, 7. Endpoints will be measured on Days -7, 2, 3, 4,and 8.

2.5 Pharmacokinetics

Plasma samples will be obtained for pharmacokinetics before dose administration and at 30 minutes, 60 minutes, 90 minutes, and 2, 4, 8, 12, 24 hours (±5 minutes at each time point), and at 48, 72, 96, 120, 144, and 168 hours (± 60 minutes at each time point) after dose administration. A PK analysis will be conducted if plasma concentrations exceed the lower limit of quantitation of 5

ng/mL using a validated analytical method. The PK analysis of ENT-03S will be conducted by using model-independent methods as implemented in WinNonlin™. The PK variables analyzed will include $AUC_{0\text{-}1h}$, $AUC_{0\text{-}2h}$, $AUC_{0\text{-}4h}$, $AUC_{0\text{-}12h}$, $AUC_{0\text{-}24h}$, AUC_{inf}, C_{max}, T_{max}, $T_{1/2}$, CL/F, V/F, and λ_z.

The PK variables are summarized as follows:

C_{max}	Maximum or peak measured plasma concentration
T_{max}	Time of maximum or peak measured plasma concentration
$t_{½}$	Apparent terminal elimination half-life; calculated as ln(2)/ λ_z. The number of data points included in the regression will be determined by visual inspection, but a minimum of three data points in the terminal phase, excluding C_{max}, will be required to estimate λ_z.
$AUC_{0\text{-}24}$	Area under the concentration versus time curve over 24 hours; calculated using linear trapezoid rule
CL/F	Clearance defined as: Dose/$AUC_{0\text{-}24\ (ss)}$.
Λ_z	Slope of the terminal elimination phase

3 STUDY DESCRIPTION

The purpose of this first in human, randomized, placebo-controlled, dose escalating study is to obtain safety, tolerability, and PK data in obese but otherwise healthy volunteers and in volunteers with obesity and Type 2 diabetes treated with a single subcutaneous dose of ENT-03S. Cohorts 1 through 5 will enroll obese but otherwise healthy volunteers. Cohorts 6 and (optionally) 7 will enroll obese subjects with Type 2 diabetes.

The study will be conducted in a Phase 1 unit. Subjects will be admitted on Day 1 and will be sent home on Day 2 after safety assessments, 24-hour PK blood samples, and other procedures (as applicable).

Sentinel dosing will be used, consisting of enrolling three subjects at a 2:1 active to placebo ratio followed by the remaining subjects in the respective dose cohorts.

Safety and pharmacokinetic data will be obtained after each cohort and escalation decisions will be made accordingly. An AUC exposure cap of 414 µg*h/mL will not be exceeded in any subject.

COHORTS 1, 2, and 3:

A screening visit will be scheduled up to 21 days in advance of dosing. Each subject will be brought to the Phase 1 unit after an overnight fast (water only after midnight) for the screening visit. After signing consent, screening procedures will be performed.

Subjects who qualify for the study and continue to meet inclusion/exclusion criteria will be brought back to the Phase 1 unit on Day -7 for placement of a CGM device and instructions on use.

On Day 1, a "sentinel" group of two active and one placebo subjects from the cohort will be admitted to the Phase 1 unit for 24 hours after an overnight fast (water only after midnight). After confirmation of inclusion/exclusion criteria and assessments including physical examination, EKG and blood tests, the CGM device will be replaced. Subjects will be administered a single subcutaneous dose of ENT-03S or placebo. PK samples will be obtained prior to dosing and at 30 minutes, 60 minutes, and 90 minutes and 2, 4, 8, 12, and 24 hours (± 5 minutes at each time point), and at 48, 72, 96, 120, 144, and 168 hours (± 60 minutes at each time point). Subjects in cohorts 1, 2, and 3 will be discharged 24 hours after the last set of assessments if there are no safety concerns. They will return to the unit after another 24 hours (48 hours post-dose) for fasting blood sampling for pharmacokinetics and to record any adverse events or safety concerns, and then again after another 24 hours (72 hours post-dose) for a third fasting blood draw and EKG, and again at 96, 120, and 144 hours post-dose for PK sampling, measurement of vital signs and weight, and an abbreviated physical exam. On Day 8 subjects will arrive at the clinic after an overnight fast (water only after midnight) for the final follow-up visit. An EKG will be performed, blood samples will be taken for PK, insulin, glucose, and other labs, the CGM will be removed, and subjects will be discharged from the study.

Once the 48-hour assessment of the first 3 "sentinel" patients has been completed, and assuming there were no safety concerns, the remaining 4 subjects in the cohort will be admitted for 24 hours after an overnight fast (water only after midnight), inclusion/exclusion criteria will be confirmed, new CGM devices will be placed, and subjects will be administered ENT-03S or placebo. Scheduled visits, assessments, and blood draws will occur as outlined for the sentinel patients.

Once all 7 subjects in the cohort have completed the trial period, the site PI and the Sponsor's CMO will review the blinded safety data. The Sponsor's CMO will then review the unblinded data, and assuming there are no concerns, advise the site PI that the next cohort can be treated.

COHORTS 4, 5, 6, and 7:

All 7 subjects in each cohort will be pre-screened and on Day -7, following confirmation of inclusion/exclusion criteria, a continuous glucose monitoring (CGM) device will be placed. Subjects will arrive after an overnight fast (water only after midnight) and after blood samples for insulin and glucose, will ingest 8 fl oz of Ensure. Blood sampling for glucose and insulin will be repeated 1 and 2-hours post-ingestion (± 5 minutes). On Day 1, a "sentinel" group of two active and one placebo subjects from the cohort will be admitted to the Phase 1 unit for approximately 26 hours after an overnight fast (water only after midnight). After confirmation of inclusion/exclusion criteria and assessments including physical examination, EKG and blood tests, the CGM will be replaced. Subjects will be administered a single subcutaneous dose of ENT-03S or placebo. PK samples will be obtained prior to dosing and at 30 minutes, 60 minutes, 90 minutes, and 2, 4, 8, 12, and 24 hours (± 5 minutes at each time point), and at 48, 72, 96, 120, 144, and 168 hours (± 60 minutes at each time point). The 24-hour PK sample and fasting blood sample will be obtained for glucose and insulin. Subjects will then be required to ingest 8 fl oz of Ensure and will have their blood drawn 1 hour and 2 hours after ingestion (± 5 minutes) to determine "post-prandial" glucose and insulin levels before being discharged from the unit. Subjects will be required to return to the unit after an overnight fast (water only after midnight) for PK sampling at 48, 72, 96, 120, 144, and 168 hours post-dose. At the 48, 72, and 168 hour visits, after blood sampling for pharmacokinetic analysis, glucose and insulin, subjects will ingest 8 fl oz of Ensure, and blood samples will be drawn for glucose and insulin levels 1 hour and 2 hours post-ingestion (± 5 minutes). The CGM device will be removed at the 168 hour/Day 8 visit and will not be replaced. Additionally, at the 168 hour/Day 8 visit, blood and urine samples will be collected for laboratory assessments and an EKG will be performed. On Day 14, subjects will return to the unit for final assessments after which the subjects will be discharged from the study.

Once the 48-hour assessment of the first 3 sentinel patients has been completed, and assuming there were no safety concerns, the remaining 4 subjects in the cohort will be admitted for 26 hours after an overnight fast (water only after midnight), inclusion/exclusion criteria will be confirmed, new CGM devices will be placed, and subjects will be administered ENT-03S or placebo. Scheduled visits, assessments, and blood draws will occur as outlined for the sentinel patients.

Once all 7 subjects in the cohort have completed the trial period, the site PI and the Sponsor's CMO will review the blinded safety data. The Sponsor's CMO will then review the unblinded data (including PK results), and assuming there are no concerns, advise the site PI that the next cohort can be treated.

All subjects will be assessed before dose administration, at 24, 48, 72, 96, 120, 144, and 168 hours after drug administration, and again at the End of Study visit. During the study, hypoglycemia and hyperglycemia will be managed according to standard measures, as per the PI's discretion.

The Investigator and/or Sponsor will interrupt this scheduled dose escalation scheme in one of following cases:

a. Experience of one severe of multiple moderate adverse events in 50% or more subjects per dose level which are judged to be probably or possibly related to study medication. This dose is considered to be the minimal intolerable dose (MID) and the dose below is then considered to be the maximum tolerable dose (MTD).
b. One or more subject(s) of the dose group experience(s) an adverse event, which may jeopardize the subject's health at Investigator's judgment, and whose relation to the study drug is considered as possibly or probably related.

3.1 Study Population

Subjects will be obese but otherwise healthy normal volunteers (cohorts 1 through 5) and obese individuals with Type 2 diabetes (cohorts 6 and 7) who fulfill the inclusion criteria.

3.1.1 Number of Patients

Approximately 49 subjects will be enrolled in this study (i.e., approximately 7 patients in each of the 7 cohorts). Note that if a patient does not complete the study, an additional subject may be included in that cohort (i.e., on the same study medication as the patient who did not complete).

3.1.2 Selection Criteria

Subjects will be selected by the PI at the Phase 1 unit. Subjects will be screened for a BMI 30-35 kg/m^2 (inclusive), HbA1c 6.5%-8.5% (cohorts 6 and 7 only), and the presence of allowed associated conditions such as hyperlipidemia, hyperglycemia, hypertension, GERD, and Type 2 diabetes (cohorts 6 and 7 only).

3.1.3 Inclusion Criteria

1. Subjects aged 18-70 years, both genders.
2. Healthy as determined by a physician, based on history, medical examination, vital signs, and laboratory tests.
3. Males that agree to use condoms for the duration of participation in the study.
4. Females of non-child-bearing potential (i.e., tubal ligation, hysterectomy, or postmenopausal).
5. Female patients of child-bearing potential with negative serum pregnancy tests and who agree to use double-barrier contraception during the study.
6. Subjects must be able to read, speak, and understand English and/or Spanish and provide written informed consent, and be willing and able to comply with study procedures.
7. Subjects must have a BMI of 30-35 kg/m^2 inclusive assessed immediately prior to screening.
8. HbA1c 6.5%- 8.5% (diabetic subjects only).
9. Subjects with Type 2 diabetes on no anti-diabetic medication or on stable doses of metformin for 4 weeks or more (diabetic cohorts only).
10. No history of active or chronic disease other than that allowed by study: hypertension, hyperlipidemia, hyperglycemia, GERD, heartburn, or Type 2 diabetes (cohorts 6 and 7 only).

3.1.4 Exclusion Criteria

1. History of excessive alcohol use (defined as $\geq$21 drinks per week for males and $\geq$14 drinks per week for females), recreational drug use within the past three months, or failure on urinary drug screen.
2. Pregnant or breastfeeding within six months of screening assessment.
3. Substantial changes in eating habits or exercise routine within the preceding three months.
4. Evidence of eating disorders.
5. $\geq$5% weight change in the past three months.
6. Bariatric surgery within the past five years.

7. Significant renal impairment (eGFR <60 mg/mL/1.73m^2).
8. Patients on anti-diabetic medications other than metformin.
9. Patients with gastroparesis.
10. Patients with Type 1 diabetes.
11. Liver function tests (i.e., ALT, AST, alkaline phosphatase bilirubin) greater than twice the upper limit of normal upon repeated measurements.
12. Diseases interfering with metabolism and/or ingestive behavior (e.g., myxedema, Cushing's disease, schizophrenia, major psychoses).
13. History of major depressive disorder within the previous two years, a lifetime history of suicide attempt, suicidal behavior within the previous month, or history of other severe psychiatric disorders.
14. Score of ≥15 on the Columbia Suicide Severity Rating Scale (C-SSRS).
15. Use of medications affecting body weight within the past three months:
    - 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
16. Any clinically significant abnormality following the Investigator's review of the physical examination and clinical laboratory tests.
17. A baseline prolongation of QT/QTc interval after repeated measurements of >450 ms; a history of unexplained syncope, cardiac arrest, unexplained cardiac arrhythmias or torsades de pointes, structural heart disease, or a family history of Long QT Syndrome (LQTS). Eligibility should be assessed using the $QTc_{F.}$value.
18. Participation in an investigational drug trial within the month prior to dosing in the present study.

3.1.5 Discontinuation Criteria

Patients may withdraw voluntarily from participation in the study at any time and for any reason.

Patients may be withdrawn on the basis of the medical monitor safety and tolerability review or the Investigator's clinical judgment, protocol deviation, change in dose of medications unrelated to study medication, or loss to follow-up.

This study may be terminated at the discretion of the Sponsor or of any regulatory agency for reasons including safety and/or efficacy.

An Investigator may elect to discontinue or stop the study at his or her site for any reason including safety.

When a patient withdraws or is withdrawn before completing the study, the date and reason for withdrawal are to be documented in the eCRF. The study site must immediately notify the study PI and the Sponsor's CMO. Patients who withdraw prematurely are to attend an early discontinuation visit, if possible, at which time they will complete all assessments.

In the event that a patient is withdrawn prematurely due to an AE or serious AE, the AE or serious AE will be followed until it resolves or stabilizes, or until it is judged by the Investigator to be no longer clinically significant.

3.2 Concomitant Medication

Beginning on Day -7 and continuing through the end of study visit, all subjects must abstain from regular use of prescribed or OTC drugs, food supplements, and herbal remedies with the exception of the following: antihypertensives, statins, antihistamines, prescription or OTC medications for heartburn and GERD, OTC pain relievers (i.e., acetaminophen, ibuprofen, naproxen), hormonal birth control, and vitamins. Subjects with Type 2 diabetes enrolled in cohorts 6 and 7 may use metformin; however, no other anti-diabetic medications are allowed.

4 MATERIALS

4.1 Study Drug

All study medication will be supplied by Enterin, Inc. as a sterile vial containing approximately 1 mL of ENT-03S at 50 mg/mL or placebo. The dose will be drawn up into a syringe by the unblinded pharmacist at the Phase 1 unit prior to use. The total dose administered will be based on the volume of liquid delivered to the subject. The volume administered to each cohort is indicated below.

Table 1: Dose and Volume of ENT-03S for Each Study Cohort

Cohort	1	2	3	4	5[1]	6[1,2]	7[2,3]
Dose (mg)	3.0	6.0	12.5	25.0	50.0	50.0	75.0
Volume (mL)	0.06	0.12	0.25	0.50	1.0	1.0	1.50

[1] Dose to be administered as 2 injections of 0.50 mL each.
[2] Obese, Type 2 diabetic cohort.
[3] Optional cohort, to be dosed if no effect on glycemia or insulin level is seen in cohort 6. Dose to be administered as 2 injections of 0.75 mL each.

4.2 Packaging and Labeling

Study medication will be supplied in labeled vials, clearly identifying the contents and indicating that the product is an investigational drug. An unblinded pharmacist at the clinical site will be responsible for drawing up the appropriate amount of study medication into a syringe and labeling the syringe for administration to the specific patient according to the randomization scheme provided by the CRO.

4.3 Storage, Dispensing and Reconciliation of Study Drug

All study medication should be stored under refrigerated conditions (2°C – 8°C) until dispensed. Storage in the Phase 1 unit should be in a locked and secure location accessible only to site staff involved with this study.

If the Phase 1 unit becomes aware that study medication has not been properly handled, the Sponsor must be contacted immediately. In such an event, study medication should not be utilized until the Sponsor provides further direction.

Neither the Investigator nor any study personnel will distribute any study medication to any person not participating in this study. The study medication will be administered at the discretion and direction of the Investigator in accordance with the conditions specified in this protocol. It is the Investigator's responsibility to ensure that accurate records of study medication issuance and return are maintained.

The Sponsor is responsible for the tracking and accountability of study medication dispensed to the Phase 1 units and will inform Phase 1 units how to return or destroy study medication once it is no longer needed at the site.

Table 2: Identification of Investigational Products

Product Name	**ENT-03S**
Dosage form	Vials with a crimped rubber gasket containing approximately 1.2 mL of 50 mg/mL sterile ENT-03S in 25% hydroxypropyl-β-cyclodextrin in phosphate buffer at pH 7.4 (Placebo will contain approximately 1.2 mL of 25% hydroxypropyl-β-cyclodextrin in phosphate buffer at pH 7.4)
Concentration (mg/mL)	50 mg/mL
Route/dosage	Subcutaneously administered ENT-03S doses will be as follows: 3.0 mg (0.06 mL) 6.0 mg (0.12 mL) 12.5 mg (0.25 mL) 25.0 mg (0.50 mL) 50.0 mg (1.0 mL) – administered as 2 injections of 0.50 mL each 75.0 mg (1.5 mL) – administered as 2 injections of 0.75 mL each (Placebo subjects will receive same volume as treatment subjects at each dosing level)
Dosing Instructions	Single dose to be administered subcutaneously to each subject

5 WARNINGS AND PRECAUTIONS

Subjects will be told that they may experience injection site discomfort and irritation. In the event of an injection site reaction, clinical staff will photograph the site reaction for inclusion in the case report file.

6 STUDY PROCEDURES

Each subject will be brought to the Phase 1 unit after an overnight fast (water only after midnight) for a screening visit up to 21 days prior to dosing. After signing consent, screening procedures, including labs, will be performed.

On Day -7, qualifying subjects will return to the Phase 1 unit for CGM device placement and instructions on use. Subjects in cohorts 4-7 will be asked to arrive after an overnight fast (water only after midnight), and after blood samples for glucose and insulin, will ingest 8 fl oz of Ensure. Repeat blood samples for blood glucose and insulin levels will be obtained 1 and 2 hours after ingestion (± 5 minutes) and subjects will then be allowed to leave the unit.

On Day 1, each subject who fulfils the inclusion/exclusion criteria will be admitted to the Phase 1 unit after an overnight fast (water only after midnight). (Note, a +1 day window is allowed between Day -7 and Day 1.) After a physical examination, body weight and EKG, fasting blood samples will be obtained. All subjects will have the CGM device replaced. The subject will then receive a subcutaneous dose of ENT-03S or placebo. PK blood samples will be obtained prior to dosing and at 30 minutes, 60 minutes, 90 minutes, and 2, 4, 8, 12, and 24 hours (± 5 minutes at each time point) and after a second examination, the subject will be discharged with the exception of subjects in the 25 mg, 50 mg, and 75 mg cohorts (Cohorts 4-7). Prior to discharge, subjects in the 25 mg, 50 mg, and 75 mg cohorts (Cohorts 4-7) will have fasting blood draws for glucose and insulin, followed by ingestion of 8 fl oz of Ensure, and repeat blood draws 1 and 2 hours (± 5 minutes) later to determine postprandial glucose and insulin levels. All subjects will return at 48, 72, 96, 120, 144, and 168 hours for PK sampling, a physical exam, and measurement of vital signs and weight. However, those subjects in the 25 mg, 50 mg, and 75 mg cohorts will arrive fasting (water only after midnight) on Days 3, 4, and 8 and will have blood drawn for fasting glucose and insulin. They will then be required to drink 8 fl oz of Ensure and have blood draws 1 hour and 2 hours (± 5 minutes) thereafter for postprandial glucose and insulin measurements. Subjects will return for a final end of study visit on Day 14 where final assessments will be performed according to the Schedule of Events, after which subjects will be discharged from the study.

6.1 Observations and Measurements

Seated vital signs (after 5 minutes rest) will be obtained at each visit.

6.2 Informed Consent

The Principal Investigator(s) at each center will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to discontinue from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

6.3 Instructions to Patients

Cohorts 1 – 3: Subjects will be instructed to come to the Phase 1 unit after an overnight fast (water only after midnight) for the Screening visit and the visits on Day 1 and Day 8. Subjects are not required to be fasted for the scheduled visits on other study days.

Cohorts 4 – 7: Subjects will be instructed to come to the Phase 1 unit after an overnight fast (water only after midnight) for all visits to the clinic.

6.4 Clinical Laboratory Procedures

See Clinical Laboratory Determination (Appendix 1) and Schedule of Events (Appendix 2) for list of laboratory procedures performed at each time point.

6.5 PK Samples

Blood samples (5.0 mL) will be collected into potassium-EDTA treated tubes for analysis of ENT-03S and its metabolite plasma concentrations. At the Sponsor's discretion, testing for inflammatory markers may also be performed on these samples.

Blood samples for PK analysis will be collected at the following times before and after dosing on Day 1: 0 (pre-dose), 30, 60, 90 minutes, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours. A sampling window of ± 5 minutes is allowed at the 1, 2, 4, 8, 12, and 24 hour time points. A sampling window of ± 60 minutes is allowed at the 48, 72, 96, 120, 144, and 168 hour time points.

Once collected, PK samples will be stored in a minus (-) 70ºC freezer until time of shipment. Labels and appropriate shipping materials will be provided to sites.

The concentration of study drug will be determined from the plasma samples using a validated analytical method. Details of the method validation and sample analysis will be included with the final clinical study report.

6.6 EKGs

12-lead EKGs will be obtained at Screening, on Day 1 prior to dosing, on Day 4, and on Day 8. The EKG machine will automatically calculate the HR and measure PR, QRS, QT and QTc intervals. Reports will be sent to a central reader for evaluation and more detailed analysis.

6.7 Additional Procedures for Diabetic Subjects (Cohorts 6 and 7)

6.7.1 In-House Glucose Testing

While subjects with Type 2 diabetes are in the clinic for in-house periods (on Days 1 and 2) and during outpatient visits (on Days -7, 3, 4, 5, 6, 7, 8, and 14), subjects will have their plasma glucose measured by a standard glucose analyzer (e.g., Yellow Spring Instruments [YSI]) for safety. Glucose will be measured at least once in the morning after an overnight fast of at least 8 hours, after the 2-hour post-prandial glucose test prior to discharge from the clinic, and whenever there are signs or symptoms of hypoglycemia or hyperglycemia.

6.7.2 Outpatient Glucose Testing

All subjects with Type 2 diabetes will be provided with a glucometer, test strips, and a diary on Day -7. Subjects will be instructed on how to use the glucometer and to obtain a glucometer reading in the fasting state (FPG) daily or any time they feel any symptoms related to hypoglycemia during the outpatient periods between Day -7 and Day 14. The subjects will be instructed to record any hypoglycemic event (<70 mg/dL) or hyperglycemic event (>240 mg/dL) in the provided diary. Additionally, the presence of symptoms that accompany hypoglycemia or hyperglycemia will be documented in the diary. Subjects will be instructed to contact the study staff in any of the following instances:

- FPG > 240 mg/dL
- The subject experiences symptoms of hypoglycemia or measures FPG < 54 mg/dL (Level 2 ADA criteria).
- The subject experiences severe hypoglycemia (Level 3 per ADA criteria).

Table 3: Classification of Hypoglycemia

Level	Glycemic Criteria/Description*
Level 1	Glucose < 70 mg/dL and ≥ 54 mg/dL
Level 2	Glucose < 54 mg/dL
Level 3	A severe event characterized by altered mental and/or physical status requiring assistance

* Definitions are based on the classification published by the American Diabetes Association (ADA) 2022.

6.8 CTCAE Definitions of Dose Limiting Adverse Events

Adverse events will be reported in accordance with the National Cancer Institute- Common Terminology Criteria for Adverse Events (CTCAE), version 5.0 (or higher). 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 non-invasive 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.

Anticipated potential adverse events are nausea and injection site irritation; these are summarized in Table 4.

Table 4: CTCAE Grading of nausea, vomiting and injection site reaction

Adverse event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Nausea	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition	Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospitalization indicated		-
Vomiting	1-2 episodes in 24 h	3-5 episodes in 24 h	≥6 episodes in 24 h; tube feeding, TPN, or hospitalization indicated.	Life-threatening consequences; urgent intervention indicated	Death
Injection site reaction	Tenderness with or without associated symptoms (e.g., warmth, erythema, itching)	Pain, lipodystrophy; edema; phlebitis	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death

6.9 Criteria for DLT Endpoints

Persistent nausea will be treated with antiemetics. Persistent injection site pain or swelling will be treated with Benadryl cream and cortisone cream (1%).

The following are the DLT endpoints:

- Severe nausea within 24 hours of taking drug
- Severe injection site reaction within 72 hours (≥Grade 3)

Additionally, an LFT elevation > 3xULN within 24 hours of ENT-03S or any AE > grade 3 within 24 hours of taking ENT-03S that is at least possibly attributable to ENT-03S will be considered dose-limiting toxicity for the purpose of analyses.

6.10 Pre-Existing Medical Conditions

All subjects enrolled in the study will be obese but otherwise healthy. Individuals with hypertension, hyperlipidemia, hyperglycemia, and GERD may be included. Cohorts 6 and 7 will

enroll obese subjects with Type 2 diabetes, on no treatment or on stable doses of metformin only for 4 weeks or more.

6.11 Treatment Emergent Adverse events

Treatment-emergent adverse event (TEAE) is defined as any event not present prior to the initiation of the treatments or any event already present that worsens in either intensity or frequency following exposure to the treatments.

AEs will be collected after signing the informed consent form and could be related or unrelated to the study drug.

AEs may be called "baseline-emergent adverse event"; this refers to any event which occurs or worsens during the staged screening process (after informed consent) including the randomization visit but prior to initiation of treatment. Separate summaries for AEs that occur prior to the initiation of the treatment versus AEs that occur after the initiation of the treatment (i.e., summary of treatment emergent adverse events) will be presented.

6.12 Laboratory Abnormalities

Clinical labs will be performed by a local laboratory. Labs to be drawn during the study include serum chemistries, a hematology panel, and urinalysis. A serum pregnancy test must be performed, and the result must be negative prior to the entry of women of childbearing potential. The Investigator must obtain verification that the local laboratory meets the standards for quality and consistency set by the College of American Pathologists.

Clinical laboratory reports must be reviewed by a physician for out-of-range values within 24 hours of receipt. Out-of-range values will be evaluated using the following notations:

- NS Not clinically significant
- LE Laboratory Error
- PT Patient abnormal; relates to the patient's usual state of health
- SG Significant, other. This value cannot be explained by any of the other flags.

By definition a lab value flagged as "SG" must be entered on the adverse clinical laboratory event page in the case report form. A laboratory test flagged "SG" must be repeated as soon as possible. The Investigator may use his/her own judgment as to whether the abnormal finding is sufficient reason to immediately withdraw the patient from the study.

If a laboratory value is considered to be serious or life-threatening the patient should be immediately discontinued from the study and appropriate therapy started. Refer to sections 6.11 and 6.12 for definition of a serious adverse event and related terms, and to sections 6.14 and 6.15 for details on reporting a serious adverse event.

6.13 AE Assessment and Recording

All adverse events, exacerbations of concomitant illnesses, or events known to be related to underlying disease processes or concomitant medications are to be recorded on the case report form throughout the study. If a pre-existing condition worsens on study, the date on which the exacerbation began should be recorded. Onset dates for study treatment-related adverse events must be on or after the date of initial study treatment use. The need to record an adverse event on the case report form is not dependent on whether the adverse event is associated with the use of the study medication. In order to avoid vague, ambiguous or colloquial expressions, the adverse event will be recorded in standard medical terminology.

Adverse event recording will include the date of onset, severity, duration, whether or not the study medication was discontinued, or its dosage changed because of the event, the treatment given and the outcome. The Investigator must also assess whether the event was related to the study medication, concurrent drug therapy, underlying disease, a combination of these factors, or if it is unknown. Patients with an adverse event should be carefully followed to determine outcome.

The Investigator will use the NCI CTCAE definitions to grade the severity of the event.

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

Grade 2: **Moderate;** minimal, local, or non-invasive intervention indicated; limiting age-appropriate 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**

The relationship or association of the study medication in causing or contributing to the adverse event will be characterized as remote, possible or probable as defined below:

Not related: Evidence indicates no plausible direct relationship to the study medication

Remote: Suggests other conditions are reasonably likely to account for the event including concurrent illness, progression or expression of the disease state, or reaction to concurrent medication

Possible: Suggests that the association of the event with the study medication is unknown; however, the adverse event is not reasonably supported by other conditions

Probable: Suggests that a reasonable temporal sequence of the event with medication administration exists and based upon the Investigator's clinical experience, the association of the event with study medication seems likely

Definite: Suggests that based upon the Investigator's experience, the association of the event with the study medication seems very certain.

Procedures such as surgery should not be recorded as adverse events. However, the medical condition for which the procedure was performed should be reported if it meets the definition of adverse event.

6.14 Reporting Requirements

Any adverse events, including both observed or volunteered problems, complaints, or symptoms that begins any time between the informed consent and the end of study visit on Day 14 are to be recorded briefly on the appropriate case report form and in detail in the source documents. A check list of adverse events may not be used during this study.

The following are specific definitions that are relevant to meeting your reporting obligations and which are included in the FDA Regulations, 21CFR Part 312.32, and International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guidelines:

Adverse Event: Any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can be any unfavorable sign (including an abnormal

laboratory finding), symptom, or disease temporally associated with the use of the investigational drug, whether or not considered related to the investigational drug.

Serious Adverse Event: An untoward event or reaction that at any dose:

- results in death
- is life-threatening
- requires inpatient hospitalization
- prolongs existing hospitalization
- results in permanent or significant disability or incapacity
- is a congenital anomaly/birth defect
- requires intervention to prevent permanent impairment/damage

Life-threatening: An event which a patient was at risk of death at the time of event.

There is a distinction between the severity and the seriousness of an adverse event. Severe is a measurement of intensity, thus a severe reaction is not necessarily a serious adverse event. For example, a headache may be severe in intensity, but would not be serious unless it met one of the criteria for serious adverse events listed previously.

6.15 Serious Adverse Events

Adverse events (AEs) and serious adverse events (SAEs) will be collected from the start of study treatment until the follow-up contact. Medical occurrences that began prior to the start of study treatment, but after obtaining informed consent were recorded on the Medical History/Current Medical Conditions CRF. The Investigator or site staff will be responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE, as provided in the study protocol,

However, any SAEs assessed as related to study participation (e.g., dosing, protocol mandated procedures, invasive tests, or change in existing therapy) or related to a concomitant medication will be recorded from the time a subject consents to participate in the study up to and including any follow-up contact.

In the event of an AE or SAE, it will be the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event and attempt to establish a diagnosis of the event based on signs, symptoms, and other clinical

information. The diagnosis was to be documented as the AE/SAE and not the individual's signs/symptoms. Once an Investigator becomes aware that an SAE has occurred in a study subject, they are' to report the information to the Sponsor within 24 hours and provide an assessment of causality.

6.16 Notification of Serious Adverse Events

Under IND regulations, 21 CFR Part 312.64, Investigators are required to notify the Sponsor promptly, within 24 hours of the sites' notification of any serious adverse events, deaths, or life-threatening problems with the investigational drug. This regulation also requires that if the adverse event is alarming, the Investigator must notify the Sponsor immediately.

The Sponsor must be notified as detailed in section 6.14, "Reporting a Serious Adverse Event." The Sponsor, in turn, will report all serious adverse events to regulatory agencies as required. In addition to the serious adverse events described in section 6.12, other events that in the Investigator's opinion suggest a significant hazard, contraindication, or precaution should be considered serious. This includes, but is not limited to, blood dyscrasias, endocrine disturbances, hemorrhage from any site, or severe skin disorder. Additional examples are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, and development of drug dependency or drug abuse. Hospitalization for elective surgery is not considered a serious adverse event. In addition, pregnancy is not a Serious Adverse Event, but is reportable and must be reported per IND regulations and timelines, 21 CFR Part 312.64, on the SAE reporting form and submitted to the Sponsor.

Patients who experience a serious adverse event must be given appropriate examinations and treatment. The Investigator must provide written information to the Sponsor as soon as possible.

When an Investigator is in doubt when to report an event, the Investigator should err on the side of caution and contact the Sponsor.

6.17 Reporting a Serious Adverse Event

Any serious adverse event, including death due to any cause that occurs during this study, whether or not believed to be related to the study medication, must be reported immediately (within one business day) via telephone/email to:

Safety@EnterinInc.com

and

Richard Larson, MD, PhD

r.larson@enterininc.com

Phone: (505) 469-2670

Specific medical questions can be addressed to:

Richard Larson, MD, PhD

Chief Medical Officer

Phone (505) 469-2670

The initial report should contain as much information as is available concerning the event in order to permit the Sponsor to file a report that satisfies regulatory requirements. Initial telephone reports of serious adverse events must be followed-up by a fax of a completed SAE report form or an appropriate event narrative. The event should also be entered into the source documents and case report form, as appropriate. When additional information is available, the Investigator should fax a follow-up SAE form or an appropriate supplementary event narrative to the Drug Safety Associate.

All appropriate serious adverse events will be reported immediately to appropriate regulatory authorities by the Sponsor. A copy of all FDA reportable serious adverse events will be mailed to all Investigators participating in ongoing clinical studies with the study medication in order to permit prompt notification of the appropriate institutional review board (IRB).

6.18 Serious Adverse Experiences: After Study Participation

Preclinical data to date do not point to specific classes of adverse events for which patients may be at risk after completion of study participation. In order to monitor for unanticipated adverse events occurring after study participation, the following must be complied with:

- Any occurrence of a patient death is to be reported any time that the Investigator becomes aware of the event.
- A congenital anomaly in an offspring of a patient where that offspring has been born after the patient used study medications should be reported.

- Any serious adverse event, for which, in the Investigator's opinion, there is a reasonable possibility that the event could have been caused by the study drug, should be reported.

6.19 Departure from Protocol for Emergency or Adverse Event

In medical emergencies, the Investigator should use medical judgment and remove the patient from immediate hazard. As soon as possible after removing the patient from hazard, the Investigator must contact the Sponsor by telephone to permit a decision as to whether the patient may continue in the study. The IRB should also be notified as to the type of emergency and the course of action. The case report form for the patient must describe the departure from the protocol and state the reason.

6.20 Vital Signs

Seated vital signs (body temperature, respiration rate, heart rate, systolic and diastolic blood pressure measurements) will be evaluated after a 5-minute rest at the visits indicated in the Schedule of Events. Body weight (without shoes) will be recorded whenever vital signs are recorded, and height (without shoes) will be recorded at screening only.

Vital sign measurements will be repeated if clinically significant or machine/equipment errors occur. Any confirmed, clinically significant vital sign measurements must be recorded as AEs.

6.21 Laboratory Assessments

Laboratory assessment samples are to be obtained at designated visits as detailed in the Schedule of Events and in Appendix 1 (Clinical Laboratory Determinations).

All clinically significant laboratory values should be captured on the CRF as AEs.

Blood and urine samples will be analyzed at the laboratory facility at the Investigational site. Urine samples will be analyzed by dipstick, and a microscopic analysis will be performed if the results of dipstick indicate abnormalities to be further investigated. All laboratory reports must be reviewed, signed, and dated by the Investigator. A legible copy of all reports must be filed with both the patient's CRF and medical record (source document) for that visit. Any laboratory test result considered by the Investigator to be clinically significant should be considered an AE (clinically significant AEs include those that require intervention). Clinically significant abnormal

values occurring during the study will be followed until repeat test results return to normal, stabilize, or are no longer clinically significant.

6.22 Physical Examinations

A complete physical examination (head, eyes, ears, nose and throat, heart, lungs, abdomen, skin, cervical and axillary lymph nodes, neurological, and musculoskeletal systems) will be performed at the Screening and Day 8 visits. Physical examinations will be performed by a physician, physician's assistant, or nurse practitioner. In addition, medical history will be recorded at screening, including smoking history, if applicable.

A limited (brief) physical examination to verify continued patient eligibility and to follow up any change in medical history will be performed at the visits indicated in the Schedule of Events. Limited physical examinations will be performed as clinically indicated at any study visit. All changes not present at baseline (Screening Visit) or described in the past medical history and identified as clinically noteworthy must be recorded as AEs.

6.23 Electrocardiogram

A 12-lead resting EKG will be obtained at Screening, prior to dosing on Day 1, on Day 4, and on Day 8. An assessment of normal or abnormal will be recorded and if the EKG is considered abnormal, the abnormality will be documented on the CRF. EKGs will be repeated if clinically significant abnormalities are observed, or artifacts are present.

6.24 Columbia – Suicide Severity Rating Scale (C-SSRS)

The C-SSRS is a questionnaire that assesses suicidal ideation and behavior using a semi- structured interview to probe subject responses. The "Baseline" version of the instrument will be administered to subjects during Screening, and the "Since Last Visit" version will be used at subsequent time points. Any subject who endorses suicidal ideation will be referred to a mental health professional for further assessment and/or treatment.

6.25 Data Safety and Monitoring Board

There will be no DSMB for this Phase 1a study. The site PI and the Sponsor's CMO will review the blinded data after the sentinel group of each cohort has been dosed and will have the option of allowing the remaining patients to be randomized to the same dosing group. The site PI and the Sponsor's CMO will also review the blinded results of clinical findings, lab results, and PKs

(where available) from each dosing group before escalating to the next dosing group. Additionally, the Sponsor's CMO will review the unblinded results at the end of each cohort.

6.26 Stopping Rules

The Sponsor's CMO and the site PI will monitor the safety of the subjects during the study and recommend changes to the protocol or temporary stopping of the trial at any time if there are significant safety concerns.

6.27 Follow-Up and Final Reports

The Investigator shall provide the Sponsor with an accurate final report within approximately 2 months after completion, termination, or discontinuation of the study. The final report may not precede retrieval of case report forms which have not been monitored.

6.28 Regulatory Aspects

Neither the Investigator nor the Sponsor shall modify this protocol without first obtaining concurrence of the other in writing. All changes must be submitted to the IRB. Protocol modifications which impact patient safety or the validity of the study must be approved by the IRB and submitted to the FDA before implementation. In the case of a medical emergency to increase safety of patients, a change may be made after discussion with the Sponsor. In these instances, the IRB and FDA will be notified as soon as possible.

7 DATA MANAGEMENT AND STATISTICS

7.1 Sample Size Considerations

Approximately 49 subjects will be enrolled in this study (i.e., approximately 7 patients in each of the 7 cohorts). Note that if a patient does not complete the study, an additional subject may be included in that cohort (i.e., on the same study medication as the patient who did not complete).

The sample size of 7 patients per cohort (i.e., 5 patients on ENT-03S and 2 on placebo) was chosen to provide an initial assessment of safety, no formal sample calculations were performed. Approximately 35 subjects on ENT-03S provides an 89% chance that an AE with an underlying probability of 7% will be seen in these patients, and 5 subjects on ENT-03S (i.e., within a cohort) provides an 83% chance that an AE with an underlying rate of 30% will be seen in the trial.

7.2 Populations for Analysis

The following are the analysis populations:

- The Screened population consists of all subjects who signed the ICF.
- The Safety Population will consist of all patients who receive at least one dose of study medication during the study with patients to be analyzed based on the actual study treatment received.
- The Pharmacokinetic (PK) Population will consist of the Safety Population subjects for whom at least one PK parameter can be derived.

7.3 Data Collection and Case Report Form Monitoring

Data reports will be generated on a weekly basis to monitor data completeness. Sites and patients who are missing data will be contacted and measures will be taken to resolve any obstacles regarding the data submission.

7.3.1 General Considerations

No formal statistical hypotheses will be tested. Descriptive summary statistics will be presented for the safety and tolerability endpoints overall. Continuous variables will be summarized using the number of observations, mean, standard deviation, median, minimum, and maximum. Categorical variables will be summarized using frequency counts and percentages. Summary statistics will be provided by cohort, pooling the placebo subjects as appropriate.

7.3.2 Safety Analyses

All safety analyses will be performed on the Safety Population.

All reported AEs will be coded using the CTCAE version 5.0 (or higher) terminology. The incidence of treatment-emergent AEs (TEAEs; events with onset dates on or after the start of the study drug) and treatment-related AEs will be summarized by system organ class (SOC) and preferred term (PT). Events with missing onset dates will be included as treatment emergent. If a patient experiences more than 1 occurrence of the same AE, the occurrence with the greatest severity and the closest association with the study drug will be used in the summary tables. SAEs and AEs that result in treatment discontinuation will be summarized. In addition, AEs of special interest will be summarized by SOC and PT; categories of AEs of special interest include nausea, vomiting and injection site reactions.

All AEs will be listed by patient, along with information regarding onset, duration, relationship and severity to study drug, action taken with study drug, treatment of event, and outcome.

Vital signs, physical examinations, EKGs, and laboratory values and changes from baseline will be summarized at each scheduled time point.

7.3.3 Pharmacokinetic Endpoints

The PK parameters described in Section 5 will be summarized using the PK population. Dose-proportionality for C_{max} and AUC_{inf} will be analyzed using an ANOVA of the natural log-transformed AUC_{inf} and C_{max}.

7.3.4 Pharmacodynamic Endpoints

Cohorts 1-3: Fasting blood glucose, leptin, insulin, insulin sensitivity, and lipids will be obtained at Screening, visit 3 (Day 1,) prior to dosing, and at visits 4 (Day 2, 24 hours post-dose), 5 (Day 3, 48 hours post-dose), 6 (Day 4, 72 hours post-dose) and 10 (Day 8, end of study).

Cohorts 4-7: Fasting blood glucose, leptin, insulin, insulin sensitivity, and lipids will be obtained at Screening, visit 3 (Day 1) prior to dosing, and at visits 6 (Day 4, 72 hours post-dose) and 10 (Day 8, 168 hours post-dose). On Day -7, Day 2, Day 3, Day 4, and Day 8, subjects will ingest 8 fl oz of Ensure and blood samples for insulin and glucose will be obtained pre-ingestion and 1 and 2 hours later.

Any improvement in these metabolic parameters will be noted. Insulin sensitivity will be assessed using the Matsuda Index and the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) using the HOMA2 calculator (https://www.dtu.ox.ac.uk/homacalculator/).

Summary statistics for the values and change from baseline will be presented for each scheduled assessment timepoint.

7.4 Interim Analysis

Blinded interim assessments of safety will be made by the site PI and the Sponsor's CMO prior to proceeding to the next dose level. Additionally, unblinded review of each cohort's data will be performed by the Sponsor's CMO at the end of each cohort.

8 ESTIMATED DURATION OF THE STUDY

This study has an estimated duration of up to 4 weeks for each patient, inclusive of the screening period. The study duration from first patient into last patient out is expected to be approximately 12 months.

9 ADMINISTRATIVE PROCEDURES AND ETHICAL CONSIDERATIONS

9.1 Medical Care

This study has an estimated duration of approximately 4 weeks per patient, inclusive of the screening period. During this period, patients will be administered medication only once on Day 1.

9.2 Patient Information and Informed Consent

The Principal Investigator(s) at each center will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. The patient must be able to read, speak, and understand English and/or Spanish in order to participate and the consent process must be conducted in the language in which the patient is proficient. Patients must also be notified that they are free to discontinue from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

The patient's signed and dated informed consent must be obtained before conducting any study Procedures.

The Principal Investigator(s) must maintain the original, signed Informed Consent Form. A copy of the signed Informed Consent Form must be given to the patient.

9.3 Study Monitoring

Before an investigational site can enter a patient into the study, a representative of the Sponsor will visit the investigational study site to:

- Determine the adequacy of the facilities
- Discuss with the Investigator(s) and other personnel their responsibilities with regard to protocol adherence, and the responsibilities of the Sponsor or its representatives. This will be documented in a Clinical Study Agreement between the Sponsor and the Investigator.

During the study, a monitor from the Sponsor or it's representative will have regular contacts with the investigational site, for the following:

- Provide information and support to the Investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the case report forms, and that investigational product accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the case report forms with the patient's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each patient (e.g., clinic charts).
- Record and report any protocol deviations not previously sent to the Sponsor.
- Confirm AEs and SAEs have been properly documented on CRFs and confirm any SAEs have been forwarded to the Sponsor and those SAEs that met criteria for reporting have been forwarded to the IRB.

The monitor will be available between visits if the Investigator(s) or other staff needs information or advice.

9.4 Audits and Inspections

Authorized representatives of the Sponsor, a regulatory authority, an Independent Ethics Committee or an Institutional Review Board may visit the site to perform audits or inspections, including source data verification. The purpose of a Sponsor audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice guidelines of the International Conference on Harmonization, and any applicable regulatory requirements. The Investigator should contact the Sponsor immediately if contacted by a regulatory agency about an inspection.

9.5 Ethics Committee Review

The final study protocol, including the final version of the Informed Consent Form, must be approved or given a favorable opinion in writing by an IRB or IEC as appropriate. The Investigator must submit written approval to the Sponsor before he or she can enroll any patient/subject into the study.

The Principal Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit patients for the study. The protocol must be re-approved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

The Principal Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational product. The Sponsor will provide this information to the Principal Investigator.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB or IEC according to local regulations and guidelines.

9.6 Standards

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements and the Sponsor's policy on Bioethics.

9.7 Confidentiality

Any research information obtained about the subject in this study will be kept confidential. A subject will not be identified by name, only by his/her initials. The subject's name or any identifying information will not appear in any reports published as a result of this study.

However, information obtained from individual subject's participation in the study may be disclosed with his/her consent to the health care providers for the purpose of obtaining appropriate medical care. The subject's medical records/charts, tests with his/her name on them may be made available to the appropriate contract research organization (CRO), the Sponsor, its potential eventual partners, and any other regulatory authorities. This is for the purpose of verifying information obtained for this study. Confidentiality will be maintained throughout the study within the limits of the law.

A subject's name will not be given to anyone except the researchers conducting the study, who have pledged an oath of confidentiality. All identifying information will be kept behind locked doors, under the supervision of the study Investigator and will not be transferred outside of the Investigator site.

A subject may take away his/her permission to collect, use and share information about him/her at any time. If this situation occurs, the subject will not be able to remain in the study. No new information that identifies the subject will be gathered after that date. However, the information about the subject that has already been gathered and transferred may still be used and given to others as described above in order to preserve the scientific integrity and quality of the study.

9.8 Protocol Adherence

The pharmacist or other designated individual will maintain records of study medications delivered to the study site, the inventory at the site, the distribution to and use by each patient, and the return of materials to the Sponsor for storage or disposal. These records should include dates, quantities, batch/serial numbers, expiration dates, in-clinic temperature log, and unique code numbers assigned to the product and study patients.

Investigator will maintain records that document adequately that the patients were provided with the correct study drug and will reconcile the products received from the drug dispensing center. Investigational product will not be returned to the Sponsor until accountability has been fully monitored.

9.9 Amendments to the Protocol

Modifications to the protocol are only possible by approved protocol amendments authorized by the study Sponsor. All protocol amendments will be approved by the appropriate regulatory authorities as well as each institutional review board prior to implementation. The Investigator must not implement any deviations from, or changes to the protocol, except where it is necessary to eliminate an immediate hazard to the study subject.

9.10 Protocol Deviations

The protocol must be read thoroughly, and the instructions followed exactly. The Sponsor and/or designee will not grant waivers for protocol deviations. Any deviation to the protocol has to be

reported as soon as possible to the Sponsor. The governing reporting guidelines for protocol deviations must be adhered to by the Investigator.

9.11 Study Termination

This study may be prematurely terminated, if in the opinion of the Investigator or the Sponsor, there is sufficient reasonable cause. Written notification documenting the reason for study termination will be provided to the Investigator or the Sponsor by the terminating party. Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Failure to enroll subjects at an acceptable rate.
- Insufficient adherence to protocol requirements.
- Insufficient complete and/or evaluable data.
- Plans to modify, suspend, or discontinue the development of ENT-03S.

Should the study be closed prematurely, all study materials must be returned to the Sponsor.

9.12 Data Handling and Record Keeping

9.12.1 Inspection of Records

The Sponsor will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, subject charts and study source documents, and other records relative to study conduct.

9.12.2 Data Management

All data relating to study procedures will be entered into electronic case report forms provided by the Sponsor. All forms must be completed electronically. All requested information must be entered on the electronic case report form. If an item is not available or not applicable this fact should be indicated by the use of "NA". Spaces should not be left blank. Electronic case report forms will be reviewed during the monitoring visits. Data will be entered into a database. Data entry and management and the production of the clinical study report will be the responsibility of the Sponsor or a designated agent.

9.12.3 Data Capture and Management

Electronic CRFs will be completed for each subject. It is the Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data entered in each subject's eCRF. Source documentation supporting the eCRF data should indicate the subject's participation in the study and document the dates and details of study procedures, AEs, and subject status.

The Investigator, or designated representative, should complete the eCRF as soon as possible after information is collected, preferably on the same day that a subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be entered immediately after the final examination. An explanation should be given for all missing data.

9.12.4 Liability and Insurance

The Sponsor has subscribed to an insurance policy covering, in its terms and conditions, its legal liability for certain injuries to participating persons arising out of this research performed strictly in accordance with the scientific protocol as well as with applicable law and professional standards.

9.12.5 Retention of Records

Study records and source documents must be preserved for at least 15 years after the completion or discontinuation of/withdrawal from the study or 2 years after the last approval of a marketing application in an ICH region, whichever is the longer time period.

The Investigator agrees to comply with all applicable federal, state, and local laws and regulations relating to the privacy of patient health information, including, but not limited to, the Standards for Individually Identifiable Health Information, 45 CFR, Parts 160 and 164 (the Health Insurance Portability Accountability Act of 1996 [HIPAA] Privacy Regulation). The Investigator shall ensure that study patients authorize the use and disclosure of protected health information in accordance with HIPAA Privacy Regulation and in a form satisfactory to the Sponsor.

9.13 Data Quality Assurance

As per GCP guidelines, the Sponsor or designee will be responsible for implementing and maintaining quality assurance and quality control systems for this study.

Participating sites, the study database, and study documentation including subject medical records may be subject to a quality assurance audit during the course of the study. In addition, inspections may be conducted by regulatory bodies at their discretion.

If sites receive a request for an inspection or written or oral inquiries regarding any aspect of the site's or Investigator's activities related to this study from a regulatory authority, the Investigator must immediately notify the Sponsor and the appropriate CRO of the request. Following this inspection and/or audit, the Investigator must notify the Sponsor of any violation or deficiency noted by the regulatory authority.

10 USE OF INFORMATION

Both the use of data and the publication policy are detailed within the clinical study agreement. Intellectual property rights (and related matters) generated by the Investigator and others performing the clinical study will be patient to the terms of a clinical study agreement that will be agreed between the Institution and the Sponsor or their designee. With respect to such rights, the Sponsor or its designee will solely own all rights and interests in any materials, data, and intellectual property rights developed by Investigators and others performing the clinical study described in this protocol, patient to the terms of any such agreement. In order to facilitate such ownership, Investigators will be required to assign all such inventions directly to the Sponsor or its designee, as will be set forth in the clinical study agreement.

11 INVESTIGATOR AGREEMENT

I have read the foregoing protocol "A First in Human, Single Center, Single Dose, Randomized, Placebo-controlled, Dose-escalating Study to Evaluate Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Subcutaneously Administered ENT-03S for the Treatment of Obesity and Diabetes" and agree to conduct the study as described therein.

Investigator's Name (Printed)

Investigator's Signature

Date

12 REFERENCES

1. Flegal KM, Kruszon-Moran D, Carroll MD, Fryar CD, Ogden CL. Trends in obesity among adults in the united states, 2005 to 2014. Jama 2016;315:2284–91. Doi:10.1001/jama.2016.6458.
2. Fontaine KR, Redden DT, Wang C, Westfall AO, Allison DB. Years of life lost due to obesity. J Am Med Assoc 2003;289:187–93. Doi:10.1016/S0140-6736(09)60318-4.
3. MacMahon S, Baigent C, Duffy S, Rodgers A, Tominaga S, Chambless L, et al. Body-mass index and cause-specific mortality in 900 000 adults: Collaborative analyses of 57 prospective studies. Lancet 2009;373:1083–96. Doi:10.1016/S0140-6736(09)60318-4.
4. Masters RK, Powers DA, Link BG. Obesity and US mortality risk over the adult life course. Am J Epidemiol 2013;177:431–42. Doi:10.1093/aje/kws325.
5. Ding M, Hu Y, Schwartz J, Koh WP, Yuan JM, Sesso HD, et al. Delineation of body mass index trajectory predicting lowest risk of??mortality in U.S. men using generalized additive mixed model. Ann Epidemiol 2016;26:698–703. Doi:10.1016/j.annepidem.2016.08.006.
6. The Global Mortality Collaboration. Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. Lancet 2016;388:776–86. Doi:10.1016/S0140-6736(16)30175-1.
7. Pi-Sunyer X. The Medical Risks of Obesity. Postgrad Med 2009;121:21–33. Doi:10.3810/pgm.2009.11.2074.The.
8. Klein S, Burke LE, Bray G a, Blair S, Allison DB, Pi-Sunyer X, et al. Clinical implications of obesity with specific focus on cardiovascular disease: a statement for professionals from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism: endorsed by the American College of Cardiology Found. Circulation 2004;110:2952–67. Doi:10.1161/01.CIR.0000145546.97738.1E.
9. The Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration (BMI Mediated Effects). Metabolic mediators of the effects of body-mass index, overweight, and obesity on coronary heart disease and stroke: A pooled analysis of 97 prospective cohorts with 1??8 million participants. Lancet 2014;383:970–83. Doi:10.1016/S0140-6736(13)61836-X.
10. Kumanyika SK. Special issues regarding obesity in minority populations. Send to Ann Intern Med 1993;119:650–4.
11. Kumunyika SK. Obesity in Minority Populations: An Epidemiologic Assessment. Obes Res 1994;2:166–82. Doi:10.1002/j.1550-8528.1994.tb00644.x.
12. Kumanyaka SK, Obarzanek E, Stevens VJ, Hebert PR, Whelton PK. Weight-loss experience of black and white participants in NHLBI- sponsored clinical trials. Am J Clin Nutr 1991;53:1631S–1638S.
13. Behan DF, Cox SH. Obesity and its Relation to Mortality and Morbidity Costs. Soc Actuar 2010:1–77.
14. http://www.who.int/diabetes/glocal-report

15. World Health Organization, Diabetes Mellitus: Report of a WHO Study Group, World Health Org., Geneva, 1985 (Tech. Rep. Ser., no. 727).
16. http://www.cdc.gov/diabetes/pubs/statsreport14/national-diabetes-report-web
17. N. Bansal, Prediabetes diagnosis and treatment: a review, World J. Diabetes 6 (2016) 296–303.
18. K. Faerch, A. Hulman, T.P. Solomon, Heterogeneity of pre-diabetes and type 2 diabetes: implications for prediction, prevention and treatment responsiveness, Curr. Diabetes Rev. 12 (2016) 30–41.
19. G. Bock, C. Dalla Man, M. Campioni, E. Chittilapilly, R. Basu, G. Toffolo, C. Cobelli, R. Rizza, Pathogenesis of pre-diabetes: mechanisms of fasting and postprandial hyperglycemia in people with impaired fasting glucose and/or impaired glucose tolerance, Diabetes 55 (2006) 3536–3549.
20. http://www.diabetesatlas.org/
21. http://gis.cdc.gov/grasp/diabetes/DiabetesAtlas.html 11
22. . S. Pugazhenthi, L. Qin, P.H. Reddy, Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease, Biochim. Biophys. Acta (2016 May 6) (pii: S0925–4439(16)30097–7).
23. . Leibson CL, Rocca WA, Hanson VA, Cha R, Kokmen E, O'Brien PC, Palumbo PJ (1997) Risk of Dementiaamong Persons with Diabetes Mellitus: A Population-based Cohort Study. American Journal of Epidemiology 145:301-308.
24. Luchsinger JA, Tang M-X, Stern Y, Shea S, Mayeux R (2001) Diabetes Mellitus and Risk of Alzheimer's Disease and Dementia with Stroke in a Multiethnic Cohort. American Journal of Epidemiology 54:635-641.
25. Profenno LA, Porsteinsson AP, Faraone SV (2010) Meta-Analysis of Alzheimer's Disease Risk with Obesity, Diabetes, and Related Disorders. Biological Psychiatry 67:505-512.
26. Wing R, Lang W, Wadden T, Safford M, Knowler W, Bertoni A, et al. Benefits of Modest Weight Loss in Improving Cardiovascular Risk Factors in Overweight and Obese Individuals With Type 2 Diabetes. Diabetes Care 2011;34:1481–6. Doi:10.2337/dc10-2415.
27. Fujioka K. Current and emerging medications for overweight or obesity in people with comorbidities. Diabetes, Obes Metab 2015;17:1021–32. Doi:10.1111/dom.12502.
28. MacDaniels JS, Schwartz TL. Effectiveness, tolerability and practical application of the newer generation anti-obesity medications. Drugs Context 2016;5:5–11. Doi:10.7573/dic.212291.
29. Myers MG, Jr., and Olson DP. Central nervous system control of metabolism. *Nature.* 2012;491(7424):357-63.
30. Arnold SE, Arvanitakis Z, Macauley-Rambach SL, Koenig AM, Wang HY, Ahima RS, et al. Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. *Nat Rev Neurol.* 2018;14(3):168-81.
31. Bence KK, Delibegovic M, Xue B, Gorgun CZ, Hotamisligil GS, Neel BG, et al. Neuronal PTP1B regulates body weight, adiposity and leptin action. *Nat Med.* 2006;12(8):917-24.

32. Sharma B, Xie L, Yang F, Wang W, Zhou Q, Xiang M, et al. Recent advance on PTP1B inhibitors and their biomedical applications. *Eur J Med Chem.* 2020;199:112376.
33. Mullard A. Phosphatases start shedding their stigma of undruggability. *Nat Rev Drug Discov.* 2018;17(12):847-9.
34. Rao MN, Shinnar AE, Noecker LA, Chao TL, Feibush B, Snyder B, et al. Aminosterols from the dogfish shark Squalus acanthias. *J Nat Prod.* 2000;63(5):631-5.
35. Lantz KA, Hart SG, Planey SL, Roitman MF, Ruiz-White IA, Wolfe HR, et al. Inhibition of PTP1B by trodusquemine (MSI-1436) causes fat-specific weight loss in diet-induced obese mice. *Obesity (Silver Spring).* 2010;18(8):1516-23.
36. Smith AM, Maguire-Nguyen KK, Rando TA, Zasloff MA, Strange KB, and Yin VP. The protein tyrosine phosphatase 1B inhibitor MSI-1436 stimulates regeneration of heart and multiple other tissues. *NPJ Regen Med.* 2017;2:4.
37. Pandey NR, Zhou X, Qin Z, Zaman T, Gomez-Smith M, Keyhanian K, et al. The LIM domain only 4 protein is a metabolic responsive inhibitor of protein tyrosine phosphatase 1B that controls hypothalamic leptin signaling. *J Neurosci.* 2013;33(31):12647-55.
38. Krishnan N, Koveal D, Miller DH, Xue B, Akshinthala SD, Kragelj J, et al. Targeting the disordered C terminus of PTP1B with an allosteric inhibitor. *Nat Chem Biol.* 2014;10(7):558-66.
39. Ahima RS, and Flier JS. Leptin. *Annu Rev Physiol.* 2000;62:413-37.
40. Takahashi N, Qi Y, Patel HR, and Ahima RS. A novel aminosterol reverses diabetes and fatty liver disease in obese mice. *J Hepatol.* 2004;41(3):391-8.
41. Pandey NR, Zhou X, Zaman T, Cruz SA, Qin Z, Lu M, et al. LMO4 is required to maintain hypothalamic insulin signaling. *Biochem Biophys Res Commun.* 2014;450(1):666-72.
42. Ahima RS, Patel HR, Takahashi N, Qi Y, Hileman SM, and Zasloff MA. Appetite suppression and weight reduction by a centrally active aminosterol. *Diabetes.* 2002;51(7):2099-104.
43. Zasloff M, Williams JI, Chen Q, Anderson M, Maeder T, Holroyd K, et al. A spermine-coupled cholesterol metabolite from the shark with potent appetite suppressant and antidiabetic properties. *Int J Obes Relat Metab Disord.* 2001;25(5):689-97.
44. Thompson D, Morrice N, Grant L, Le Sommer S, Lees EK, Mody N, et al. Pharmacological inhibition of protein tyrosine phosphatase 1B protects against atherosclerotic plaque formation in the LDLR(-/-) mouse model of atherosclerosis. *Clin Sci (Lond).* 2017;131(20):2489-501.
45. Ricke KM, Cruz SA, Qin Z, Farrokhi K, Sharmin F, Zhang L, et al. Neuronal Protein Tyrosine Phosphatase 1B Hastens Amyloid beta-Associated Alzheimer's Disease in Mice. *J Neurosci.* 2020;40(7):1581-93.
46. Perni M, Flagmeier P, Limbocker R, Cascella R, Aprile FA, Galvagnion C, et al. Multistep Inhibition of alpha-Synuclein Aggregation and Toxicity in Vitro and in Vivo by Trodusquemine. *ACS Chem Biol.* 2018;13(8):2308-19.

47. Smith AM, Maguire-Nguyen KK, Rando TA, Zasloff MA, Strange KB, and Yin VP. The protein tyrosine phosphatase 1B inhibitor MSI-1436 stimulates regeneration of heart and multiple other tissues. NPJ Regen Med. 2017;2:4.
48. Ellis J. Multiple Doses of Trodusquemine Improve Glucose Tolerance in Type 2 Diabetic Subjects. 2071-PO (Abs), 69th Scientific Sessions American Diabetes Association 2009
49. Venema W, Severi I, Perugini J, Di Mercurio E, Mainardi M, Maffei M, et al. Ciliary Neurotrophic Factor Acts on Distinctive Hypothalamic Arcuate Neurons and Promotes Leptin Entry Into and Action on the Mouse Hypothalamus. *Front Cell Neurosci.* 2020;14:140.
50. Ladyman SR, and Grattan DR. JAK-STAT and feeding. *JAKSTAT.* 2013;2(2):e23675.

13 APPENDIX 1: CLINICAL LABORATORY DETERMINATIONS

<table>
<tr><th colspan="3">Blood Tests</th></tr>
<tr><th>Hematology</th><th>Serum Chemistry</th><th>Pharmacodynamic Labs</th></tr>
<tr><td>Full and differential blood count
Hematocrit (Hct)
Hemoglobin (Hb)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Mean corpuscular volume (MCV)
Platelet count
Red blood cell (RBC) count
White blood cell (WBC) count with differential</td><td>Albumin
Alanine aminotransferase (ALT)
Alkaline phosphatase (ALP)
Aspartate aminotransferase (AST)
Blood Urea Nitrogen (BUN) or Urea
Bicarbonate (HCO_3)
Creatinine
Creatine kinase and subtypes
Electrolytes (Na, K, Cl, Ca, P)
Gamma—glutamyl transpeptidase (GGT)
Fasting blood glucose
Human chorionic gonadotropin (HCG) (pre-menopausal females only)
Lactate dehydrogenase (LDH)
Total bilirubin
Direct bilirubin
Total cholesterol, LDL, HDL, VLDL cholesterol,
Triglycerides, FFA
Insulin
Leptin
Insulin sensitivity</td><td>Fasting blood glucose
Leptin
Fasting Insulin
Insulin sensitivity
Lipids</td></tr>
<tr><td colspan="3">Mixed Meal Tolerance Testing (for cohorts 4 – 7): Fasting glucose and insulin measured followed by ingestion of 8 oz. of Ensure with repeat blood draws at 1 and 2 hours (+ 5 mins) later with postprandial glucose and insulin measured. Insulin resistance should be assessed using the Matsuda Index and the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) using the HOMA2 calculator.</td></tr>
<tr><td colspan="3">Urine Dipstick: pH, Protein, Glucose, Ketone bodies, Indicators of blood and WBCs, Specific gravity and Urobilinogen</td></tr>
<tr><td colspan="3">Coagulation: Prothrombin time (PT), Activated partial thromboplastin time (PTT)</td></tr>
<tr><td colspan="3">HIV/Hep B Antigen tests (inclusion #2): HIV 1/0/2 Antigen/Antibody (4th Generation), HIV ½ Ab differentiation (reflex), HIV RNA Qualitative PCR (reflex) HBsAg, HCV Antibody, HCVNAA</td></tr>
<tr><td colspan="3">Pregnancy test: A serum pregnancy test will be performed on all female patients of child-bearing potential at Screening and on Day 8. A urine pregnancy test will be performed on all female patients of child-bearing potential on Day 1 prior to dosing.</td></tr>
</table>

14 APPENDIX 2: SCHEDULE OF EVENTS FOR EACH COHORT

Schedule of Events	Screening (Visit 1)	Day -7 (Visit 2)	Day 1* (Visit 3)	Day 2 (Visit 4, 24 hrs)	Day 3 (Visit 5, 48 hrs)	Day 4 (Visit 6, 72 hrs)	Day 5 (Visit 7, 96 hrs)	Day 6 (Visit 8, 120 hrs)	Day 7 (Visit 9, 144 hrs)	Day 8 (Visit 10, 168 hrs)	Day 14 (Visit 11, EOS)
Consent	x										
Demographics	x										
Inclusion/exclusion	x	x	x								
Medical history	x										
Physical exam[1]	x	x	x	x	x	x	x	x	x	x	x
Vital signs (seated)	x	x	x	x	x	x	x	x	x	x	x
Body weight	x	x	x	x	x	x	x	x	x	x	x
Waist circumference	x										x
Drug screen	x		x								
Serum chemistry[2]	x				x					x	
Pharmacodynamic labs[2]	x					x				x	
FBS[3]	x	x[3]	x	x	x	x				x[3]	
HbA1C	x										
Post-prandial glucose[3]		x		x	x	x				x	
Post-prandial insulin[3]		x		x	x	x				x	
CGM start		x	x[4]								
CGM stop			x							x	
Hematology	x					x				x	
Urinalysis	x					x				x	
EKG	x		x			x				x	
Serum pregnancy test	x									x	
Urine pregnancy test			x								
YSI Glucose testing[5]		x	x	x	x	x	x	x	x	x	x
Adverse events			x	x	x	x	x	x	x	x	x
PK samples[6]			x	x	x	x	x	x	x	x	
C-SSRS[7]	x					x				x	
Randomization			x								

Schedule of Events	Screening (Visit 1)	Day -7 (Visit 2)	Day 1* (Visit 3)	Day 2 (Visit 4, 24 hrs)	Day 3 (Visit 5, 48 hrs)	Day 4 (Visit 6, 72 hrs)	Day 5 (Visit 7, 96 hrs)	Day 6 (Visit 8, 120 hrs)	Day 7 (Visit 9, 144 hrs)	Day 8 (Visit 10, 168 hrs)	Day 14 (Visit 11, EOS)
Schedule visit days	x										
Administer ENT-03S or Placebo			x								
Dispense glucometer + diary[8]		x									
Collect glucometer + diary[8]											x
Check for injection site reaction[9]			x	x		x					
Record Medications	x	x	x	x	x	x	x	x	x	x	x

* A +1 day window is allowed between Day -7 and Day 1.

[1] Complete physical exams will be performed at the Screening and Day 8 visits. For all other visits, abbreviated physical exams will be performed.

[2] See Appendix 1 for list of tests included in Serum Chemistry and Pharmacodynamic Lab panels.

[3] On Day -7, Day 2, Day 3, Day 4, and Day 8, after fasting glucose and insulin at time 0, subjects in the 25 mg, 50 mg and 75 mg cohorts (Cohorts 4-7) will be given 8 fl oz of Ensure, and blood draws for insulin and glucose will be repeated 1 and 2 hours later.

[4] All subjects will have the CGM replaced on Day 1.

[5] YSI glucose testing to be performed on Type 2 diabetic subjects (cohorts 6 and 7) only.

[6] PK samples will be taken at time 0 and at 30, 60, and 90 minutes and 2, 4, 8, 12, 24 48, 72, 96, 120, 144, and 168 hours following dosing. A window of ± 5 minutes is allowed for the 0, 30 minute, 60 minute, and 90 minute and the 2, 4, 8, 12, and 24 hour time points. A window of ± 60 minutes is allowed for the 48, 72, 96, 120, 144, and 168 hour time points.

[7] The "Baseline" version of the C-SSRS will be administered to subjects during Screening, and the "Since Last Visit" version will be used at subsequent time points.

[8] Subjects in cohorts 6 and 7 only.

[9] Subjects will be assessed for injection site reactions at 12, 24, and 72 hours.