STATISTICAL ANALYSIS PLAN
ENT-01-30

A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Multiple Dose Study to Evaluate Safety, Tolerability and Efficacy or Orally Administered ENT-01 for the Treatment of Parkinson's Disease-Related Constipation (KARMET)

Protocol Number:	ENT-01-30
Name of Test Drug:	ENT-01
Phase:	2b
Methodology:	Double-Blind, Placebo-Controlled, Randomized Trial
Sponsor:	Enterin Inc. 2005 Market Street, Philadelphia, PA 19103
Sponsor Representative:	Denise Barbut, MD FRCP President and CMO
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SIGNATURE PAGE

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Sponsor Approval

By signing this document, I acknowledge that I have read the document and approve of the planned statistical analyses described herein. I agree that the planned statistical analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the statistical methodology described in the protocol, clinical development plan, and all applicable regulatory guidances and guidelines.

I have discussed any questions I have regarding the contents of this document with the biostatistical author.

I also understand that any subsequent changes to the planned statistical analyses, as described herein, may have a regulatory impact and/or result in timeline adjustments. All changes to the planned analyses will be described in the clinical study report (CSR).

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REVISION HISTORY

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ABBREVIATIONS

Abbreviation	Definition of Terms
AE	Adverse Events
ADL	Activities of Daily Living
ANCOVA	Analysis of Covariance
ATC	Anatomic Therapeutic Class
BDI	Beck Depression Inventory
CMH	Cochran-Mantel-Haenszel
CSBM	Complete Spontaneous Bowel Movement
CSR	Clinical Study Report
C-SSRS	Columbia Suicide Severity Rating Scale
DLT	Dose limiting Toxicity
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ENS	Enteric Nervous System
EOS	End of Study
ET	Early Termination
GI	Gastrointestinal
ICH	International Conference on Harmonization
ID	Identification
IRT	Interactive Response Technology
IXRS	Interactive voice/web-response system
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed Model Repeated Measures

Abbreviation	Definition of Terms
MMSE	Mini Mental State Examination
mITT	Modified Intention to Treat
NCI-CTCAE	National Cancer Institute- Common Terminology Criteria for Adverse Events
PAC-QoL	Patient Assessment of Constipation Quality of Life
PAC-SYM	Patient Assessment of Constipation Symptoms
PD	Parkinson's Disease
PK	Pharmacokinetics
PT	Preferred Term
QOL	Quality of Life
RASMET	Evaluation of Safety and Tolerability of ENT-01 for the Treatment of Parkinson's Disease Related Constipation
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAPS-PD	The Scale for the Assessment of Positive Symptoms -Parkinson's Disease
SBM	Spontaneous Bowel Movement
SD	Standard Deviation
SOC	System Organ Class
TEAEs	Treatment Emergent Adverse Events
ULN	Upper limit of normal
UPDRS	Unified Parkinson's Disease Rating Scale
US	United States
WHO	World Health Organization

Abbreviation	Definition of Terms
WHO-DD	World Health Organization Drug Dictionary

1. INTRODUCTION AND OBJECTIVES OF ANALYSIS

1.1. Introduction

Constipation is a common problem worldwide, affecting 2% to 27% of the population, with most estimates varying from 12% to 20% (Bouras and Tangalos, 2009). The prevalence of constipation increases to 30%-40% among people aged >65 years and women are disproportionately affected. Constipation is much more common among patients with Parkinson's Disease (PD) than in the general population. There are 1 million people suffering from Parkinson's Disease in the US, of which roughly 60%, or 600,000 suffer from chronic constipation and in most, the condition is chronic, severe and unresponsive to standard therapy (Ondo et al., 2012; Zangaglia et al., 2007). Constipation not only constitutes a major economic burden, but it also significantly affects the quality of life of the individual with Parkinson's disease. An effective prokinetic medication for individuals with PD would be a useful addition to the currently available treatments for this disabling condition.

The individual with Parkinson's Disease suffers from a form of constipation that is believed to be caused principally by delayed transit through the colon. In addition, defecation is often impaired by dysfunction of the Parkinson's Disease subject's anorectal reflex.

The pathophysiology of the gastrointestinal (GI) dysfunction in Parkinson's Disease involves deposition of alpha-synuclein within both the enteric nervous system (ENS) as well as within the brainstem. For reasons that remain unknown alpha-synuclein, which is a protein normally produced in neurons, forms neurotoxic intracellular aggregates in Parkinson's Disease. Squalamine is a small molecule, originally discovered in the liver of the dogfish shark, where it is delivered following a meal via the biliary tract into the intestine (Moore et al., 1993). In a recent study, squalamine has been shown to prevent the formation of toxic alpha-synuclein aggregates (Perni et al., 2017). Squalamine both excites the neurons of the ENS and protects them from alpha-synuclein induced cytotoxicity. We will evaluate squalamine as a therapeutic to treat the constipation associated with Parkinson's Disease and we will explore its effect on sleep architecture and hallucinations/delusions.

A Phase 2a study, *Evaluation of Safety and Tolerability of ENT-01 for the Treatment of Parkinson's Disease Related Constipation (RASMET)* was conducted. The purpose of the study was to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of an orally administered medication to relieve symptoms of constipation associated with Parkinson's Disease. The results of the study indicated that orally administered ENT-01 was safe and significantly improved bowel function and neurologic symptoms**.** Minimal systemic absorption suggests that improvements result from local stimulation of the ENS. Data from this study informed the development of the 2b study.

1.2. Objectives of Statistical Analysis

This statistical analysis plan (SAP) is designed to outline the methods to be used in the analysis of study data in order to answer the study objectives. Populations for analysis, data handling rules, statistical methods, and formats for data presentation are provided. The statistical analyses and summary tabulations described in this SAP will provide the basis for the results sections of the clinical study report (CSR) for this trial.

1.2.1 Primary Objective

The primary objective is to evaluate the safety, tolerability, and efficacy of repeated oral doses of ENT-01 for up to 25 days in subjects with Parkinson's Disease related constipation in a randomized, double-blind, placebo-controlled study.

- **Primary Safety Objective**: The primary safety objective of this study will be evaluation of the safety of repeated daily oral doses of ENT-01 for up to 25 days in patients with PD- related constipation.
- **Primary Tolerability Objective**: The primary tolerability objective will be evaluation of recurrent vomiting, recurrent diarrhea, abdominal pain and dizziness.
- **Primary Efficacy Objective**: The primary efficacy objective will be evaluation of the efficacy of repeated daily oral doses of ENT-01 for up to 25 days in improving CSBM rate.

1.2.2 Secondary Objectives

The secondary objectives include:

- To determine if the correct starting dose (75mg or 150mg) can be predicted from the baseline weekly CSBM rate.
- To collect information on the effect of ENT-01 on CSBM, SBM, ease of passage, consistency, suppository/enema use and subjective bowel function and quality of life (QOL).

1.2.3 Exploratory Objectives

The exploratory objectives include:

- To determine the decrease in efficacy in the blinded placebo withdrawal and washout periods.
- To collect information on the effect of ENT-01 on motor and non-motor symptoms of Parkinson's disease including mood, memory, sleep, and hallucinations/delusions.
- To collect information on the effect of ENT-01 on levodopa absorption.
- To collect information on the effect of ENT-01 on the stool microbiome.

2. STUDY DESIGN

2.1. Synopsis of Study Design

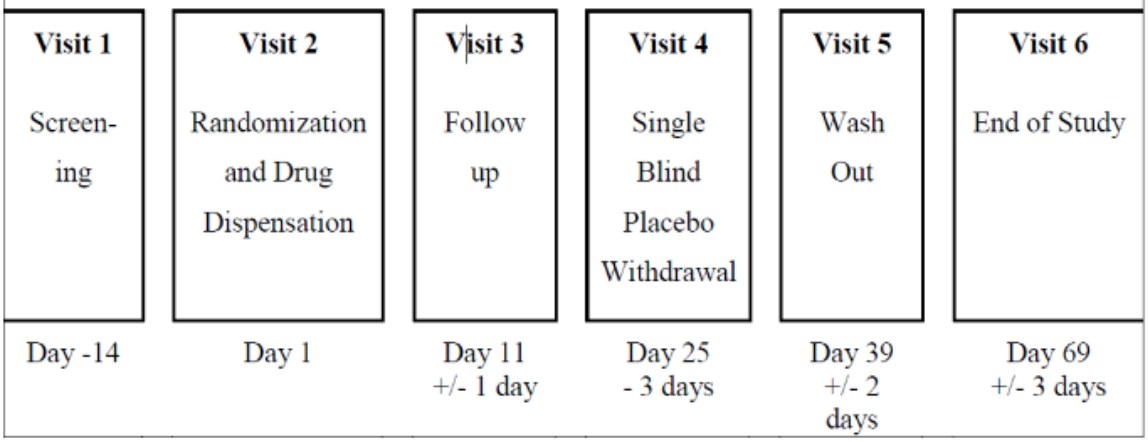
This study will be conducted as a multicenter, randomized, double-blind, placebo-controlled study in patients with Parkinson's Disease and constipation.

This study has two parts. The first part of this randomized approximately 72 subjects in a 3:1 to treatment or placebo ratio (double-blind), with approximately 54 subjects allocated to the treatment groups and approximately 18 to placebo. In the second part of the study, an additional 80 patients (approximately) will be randomized 1:1 to treatment or placebo (double-blind) with approximately 40 subjects allocated to each group.

In both parts of the study, the study was conducted as a multicenter, randomized, double-blind, placebo-controlled study. A subject's total study duration was no more than 10 weeks and total active dosing days was a maximum of 25 days.

During the active dosing period, patients will be able to increase/adjust their dose every three days. This is called the Dose Adjustment period. After the subject documents two complete spontaneous bowel movements in three days, the subject will fix at the dose for the remainder of the study. This is called the Fixed Dose period. After completing up to 25 days of dosing, all subjects will be given placebo for 2 weeks (single-blind) followed by 4 weeks of wash-out.

Figure 1. Visit Schedule Overview



2.2. Randomization Methodology

Randomization occurs after all screening assessments have been completed and after the Investigator has verified that they are eligible. In the first part of the study, patients will be

randomized 3:1 to receive either ENT-01 or placebo; in the second part of the study patients are randomized 1:1 to receive either ENT-01 or placebo. Any patient identification numbers that are assigned will not be reused, even if the patient does not receive treatment. The patient's identification (ID) number will be used on that patient's eCRFs and serious adverse event (SAE) forms.

Randomization will be conducted centrally via an Interactive voice/web-response system (IXRS). The randomization will be stratified by the baseline weekly CSBM rate. Subjects with a baseline CSBM rate of 0-0.9 will begin treatment with 150 mg of ENT-01 or 6 tablets of placebo and Subjects with a baseline CSBM rate of 1.0-2.9 will begin with 75 mg of ENT-01 or 3 tablets of placebo. Note that in the second part of the study, the baseline CSBM criteria of 1.0 – 2.9 was modified to 1.0 – 3.0 (i.e., including subjects with a baseline CSBM rate of 3.0).

Instructions for patient screening, registration, number assignment, and enrollment are referenced in the Interactive Response Technology (IRT) vendor manual.

2.3. Stopping Rules and Unblinding

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

It is anticipated that no more than 2 subjects out of 60 (3.3%) will have an AE of grade 4 or 5 that is at least possibly related to ENT-01 in this Study. Should there be more than 2 subjects with an AE grade 4 or 5 that is at least possibly related to ENT-01 in the cohort of subjects randomized to ENT-01, the study will be put on an immediate clinical hold.

In addition, individual safety stopping criteria will include:

- Reaching DLT before a prokinetic effect
- Having a non-DLT gastrointestinal adverse event > grade 3 within 24 hours of taking ENT-01 that is at least possibly attributable to ENT-01
- A fall in systolic blood pressure to < 80 mm Hg upon rising from lying to sitting or standing
- A fall in diastolic blood pressure to < 40 mm Hg upon rising from lying to sitting or standing
- Elevation of liver function tests (LFTs) > 3 times the upper limit of normal (ULN)

A treatment assignment should only be unblinded in a situation of urgent medical necessity, when the identity of the study medication must be known in order to select continuing therapy for the disease under study or an AE. The decision should be made only after consultation with the unblinded Medical Monitor, unless the urgency of a case requires immediate action. Treatment assignments can be obtained through a controlled transaction with IXRS. If a treatment code is unblinded for any reason, the Investigator will notify Sponsor within 24 hours and will document the following in the eCRF: who unblinded the code, the reason for doing so, and the date of unblinding.

2.4. Study Procedures

The schedule of events, as outlined in the study protocol, is provided in Table 1.

Table 1. Schedule of Events

	Visit 1 Screening	Visit 2 Randomization	Visit 3 Follow-Up	Visit 4 End of Treatment	Visit 5 End of Placebo	Visit 6 End of Study	Early Termination
	Day -14	Day 1 (+/-2days)	Day 11 (+/- 1day)	Day 26 (- 2 days)	Day 39 (+/- 2days)	Day 69 (+/- 3 days)	
Informed Consent	X						
Inclusion/Exclusion Criteria [a]	X	X					
Demographics/Medical History	X						
Complete Physical Exam	X						X
Brief Physical Exam			X	X	X	X	
Height	X						
Weight	X	X	X	X	X	X	X
Orthostatic Vital Signs[b]	X	X	X	X	X	X	X
12-lead ECG	X	X	X	X	X	X	X
Labs and Urinalysis	X		X	X	X	X	X
Pregnancy Testing[c]	X	X	X	X	X	X	X
In-clinic IP/Placebo Dosing[d]			X	X	X		
In-clinic Levodopa Dosing		X	X	X	X	X	X
Levodopa PK Draws[e]		X	X	X	X	X	X
IP/Placebo Dispense		X	X	X			
IP/Placebo Collection and Accountability			X	X	X	X	X

	Visit 1 Screening	Visit 2 Randomization	Visit 3 Follow-Up	Visit 4 End of Treatment	Visit 5 End of Placebo	Visit 6 End of Study	Early Termination
	Day -14	Day 1 (+/- 2 days)	Day 11 (+/- 1 day)	Day 26 (- 2 days)	Day 39 (+/- 2 days)	Day 69 (+/- 3 days)	
Instructions to Discontinue Laxatives and other BM Medications	X						
Dispense Rescue Medications[f]	X	X	X	X	X		
Stool Diary Instruction/Review[g]	X	X	X	X	X	X	X
AE Review	X	X	X	X	X	X	X
Prior and Con Meds	X	X	X	X	X	X	X
Rome IV questionnaire	X						
MDS-UPDRS[h]	X		X	X	X	X	X
C-SSRS	X	X	X	X	X	X	X
BDI-II	X		X	X	X	X	X
MMSE	X		X	X	X	X	X
SAPS-PD, Hallucination/Delusion Questionnaire[i]	X	X	X	X	X	X	X
PAC-QOL and PAC-SYM		X	X	X	X	X	X
Dispense Stool Collection Kits	X		X				
Collect Stool Sample[j]		X		X			

a. Confirmation of eligibility: To be randomized to receive double-blind study medication in this study, subjects must meet the following criteria:
- Have had at least 11 "Available Data Days" (days with electronic diary compliance sufficient for determining whether a BM occurred)
- Have had <3 CSBMs/week based upon the average CSBM rate during the first 2 screening weeks (baseline period).
- Be approved for randomization by the enrollment committee

b. Three sets will be taken. Time points to be defined. Orthostatic vital signs will include supine BP and HR, sitting or standing BP and HR, RR, and body temperature. Supine measurements will be taken after the subject has been in the supine position for at least 5 minutes. Sitting or standing measurements will be taken within 5 minutes of sitting or standing from the supine position. Respiration rate and body temperature will be taken after the subject has been sitting or standing for at least 5 minutes.

c. For women of child-bearing potential, on-site with urine pregnancy test kit. If the on-site UPT is positive, a serum pregnancy test will be done. For post- menopausal women <60 years or of age, a serum FSH will be done at the screening visit only.

d. Sites will use a centralized computer-based system to obtain the randomization bottle(s) assigned to each subject.
- Subjects with baseline CSBMs from 0 to 0.9 will be randomized to begin treatment with ENT-01 150 mg or 6 tablets of placebo.
- Subjects with baseline CSBMs from 1.0 to 3.0 will be randomized to begin treatment with ENT-01 75 mg or 3 tablets of placebo.

- Subjects will self-administer study medication at home. The dose will be taken upon awakening, on an empty stomach with 8 oz. of water.
- Subjects will be advised to stay at home for 4 to 6 hours after taking the first dose of medication in case there is a need to evacuate urgently. After the first dose, subjects will know if and when to leave the house.

e. A subset of 20 subjects from this study population will participate in an L-Dopa PK Study, as follows:
- Visits 2 and 6: Blood draws for L-dopa levels (PK) will be drawn at Time zero.
- The first daily dose of L-Dopa will be administered on site after the first blood draw. There will be another blood draw at 20, 40, and 60 minutes post- L-Dopa dose
- Visits 3. 4, and 5: Immediately upon arrival at the study site, the subject will take study medication (ENT-01 or placebo) on an empty stomach. Blood will be drawn at Time zero. 30 to 60 minutes after taking ENT-01 or placebo, the subject will take their first daily dose of L-Dopa. Blood will be drawn again 20, 40, and 60 minutes post-L-Dopa dose. The subject may have food 60 minutes after taking ENT-01.

f. Rescue medications will be dispensed as needed and instructions given concerning laxatives and other medications to treat constipation.

g. Subjects will be educated concerning use of the electronic diary to report stools immediately after a bowel movement

h. The first 3 parts of the MDS-UPDRS should be administered while the subject is in an ON state, approximately one hour after taking L-Dopa (if applicable). In some cases, patients will reach the “on” state earlier or later than at 60 minutes. In those cases, MDS-UPDRS should be performed when the patient is “on”.

i. Those subjects who have a SAPS-PD score >0 on Visits 1 and 2 will have the SAPS-PD and hallucination/delusion questionnaire at all subsequent visits.
- If only Visit 1 or Visit 2 has a score >0, then SAPS-PD will not be repeated in subsequent visits.

j. The stool sample should be collected during the EoT visit if the subject does not wish to return for the Washout Visit.

2.5. Study Periods

The study consists of the following periods:

1. **Screening Period**: This period lasts from Visit 1 to the day before Visit 2 (Day of randomization)
2. **Treatment Period**: This period lasts from Visit 2 (day of randomization) to the day before Visit 4 (start of single blind placebo period) and is divided into two sub-periods
   - 2.1. **Dose Escalation Period**: This period starts at Visit 2 and ends when the subject is on a Fixed Dose of study medication.
   - 2.2. **Fixed Dose Period**: This period starts when the subject is on a Fixed Dose of study medication and lasts until Visit 4. The fixed dose period is only defined if the subject was in the treatment period for at least seven days after the date of randomization. In the event that a subject is on the same dose until the start of the single blind placebo period or until withdrawal from treatment after being in the treatment period for at least seven days after randomization, then the fixed dose period will be the entire treatment period and the dose escalation period will not be defined. If the subject was not on their fixed dose for at least seven days, then the fixed dose period will extend to the subject's last seven days of treatment (including fixed dose) in order to derived rate variables during the fixed dose period, as described in Section 4.7. Missing diary entries in the middle of treatments of the same dose are not considered when determining the start of the fixed dose period.
3. **Single Blind Placebo Withdrawal Period**: This period starts at Visit 4 and ends at Visit 5. Weekly rate derivations during the single blind placebo withdrawal period will not include assessments on the Visit 4 date.
4. **Wash Out Period**: This period starts at Visit 5 and ends at Visit 6.

2.6. Efficacy, Pharmacokinetic, Safety and Tolerability Endpoints

2.6.1 Efficacy Endpoints

Primary Efficacy Endpoint

The primary efficacy endpoint is the change from baseline in the weekly CSBM rate during the Fixed Dose period.

Baseline is defined as the weekly CSBM rate during the 2-week screening period. If a subject has more than two weeks of entries during the screening period, consecutive missing entries during the screening period will be excluded from baseline calculations, and the last fourteen days with entries during the screening period will be used to derive baseline weekly rates.

Subjects with fewer than 11 days of diary data during screening would be excluded from the study. The primary efficacy endpoint will be obtained as the weekly CSBM rate during the Fixed Dose period with exceptions as noted in section 4.7. The frequency of CSBMs will be

determined via a web-based electronic diary where the passage of a bowel movement or absence thereof will be recorded daily throughout the study by the subject.

2.6.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints include:

- Frequency of dose adjustments in the Dose Adjustment period: The number of dose adjustments a subject has from the initial starting dose to the subject's fixed dose.
- Prokinetic bowel response during the Fixed Dose period: A prokinetic response is defined as a weekly CSBM rate increase of 1 or more compared to baseline or an absolute rate of 3 or more CSBMs per week.
- Change from baseline in the weekly SBM rate during the Fixed Dose period: An SBM is a bowel movement which did not occur within 24 hours after taking rescue medication.
- SBM bowel response to ENT-01 during the Fixed Dose period: An SBM bowel response is defined as a weekly SBM rate increase of 1 or more compared to baseline or an absolute rate of 3 or more SBMs per week.
- Change from baseline in stool ease of passage during the Fixed Dose period: Stool ease of passage is rated on a 1-7 ordinal point scale (see APPENDIX 4 of the Protocol). A subject's ease of passage score for a given time period is the average of the ease of passage scores associated with SBMs during that time period.
- Change from baseline in stool consistency during the Fixed Dose period: Stool consistency is measured using the 7-point Bristol Stool Chart (see APPENDIX 5 of the Protocol). A subject's stool consistency score for a given time period is the average of the stool consistency scores associated with SBMs during that time period.
- Change from baseline in suppository/enema use during the Fixed Dose period: Suppository/enema use is measured as a weekly average during the Fixed Dose period. This is captured in the subject stool diary.
- Time to first CSBM in the Fixed Dose period: Time to first CSBM in the Fixed Dose period is defined as the time from the subject taking the first dose of study medication in the Fixed Dose period until the subject has a CSBM.
- Time to first SBM in the Fixed Dose period: Time to first SBM in the Fixed Dose period is defined as the time from the subject taking the first dose of study medication in the Fixed Dose period until the subject has an SBM.
- Time to first rescue medication use in the Fixed Dose period: Time to first rescue medication use in the Fixed Dose period is defined as the time from the subject taking the first dose of study medication in the Fixed Dose period until the subject first uses rescue medication.
- Stool-related subjective assessments using the PAC-SYM: Subjective bowel function data is captured using the Patient Assessment of Constipation-Symptoms: The PAC-SYM is a 12-item questionnaire, and each question has a 0 (Absent) – 4 (Very Severe) point response scale. The PAC-SYM overall score is the score obtained by averaging the response from the 12 items. The PAC-SYM scores and change from baseline will be calculated for each scheduled assessment time-point.

- Stool-related subjective assessments using the PAC-QOL: Constipation-related quality of life is assessed using the Patient Assessment of Constipation-Quality of Life. The PAC-QOL is a 28-item questionnaire, and each question has a 5-point ordinal response scale. The PAC-QOL endpoints consist of an overall average score and the four subscale scores (i.e., physical discomfort, psychosocial discomfort, worries/concerns, and satisfaction). The PAC-QOL scores and change from baseline will be calculated for each scheduled assessment time-point.

2.6.3 Exploratory efficacy endpoints

The exploratory efficacy endpoints include:

- A weekly CSBM rate increase of 1 or more compared to baseline and an absolute rate of 3 or more CSBMs per week in the Fixed Dose period (i.e., this is a modification of the Prokinetic Response secondary endpoint using "AND" instead of "OR" as the criterion).
- A weekly SBM rate increase of 1 or more compared to baseline and an absolute rate of 3 or more CSBMs per week in the Fixed Dose period.
- Change from baseline in the weekly CSBM rate during the Placebo Withdrawal and Washout periods.
- Change from baseline in the weekly SBM rate during the Placebo Withdrawal and Washout periods.
- Change from baseline in stool ease of passage during the Placebo Withdrawal and Washout periods.
- Change from baseline in stool consistency during the Placebo Withdrawal and Washout periods.
- Change from baseline in suppository/enema use during Placebo Withdrawal and Washout periods.
- Improvement in frequency and/or severity of hallucinations/delusions during the Fixed Dose period over baseline: Improvement is defined as an improvement of 2.33 points or greater reduction in score from baseline on the Scale for the Assessment of Positive Symptoms -Parkinson's Disease (SAPS-PD). Up to 40% of subjects are expected to have minor or major hallucinations and/or delusions. The SAPS-PD will be administered at Screening, and subsequently will only be administered to those subjects with hallucinations and/or delusions (as determined by an SAPS-PD score >0 at the Screening visit).
- Improvement of the symptoms of Parkinson's Disease as assessed by the UPDRS: The standard four-part UPDRS will be administered to determine whether there is any deterioration in overall scores during the treatment period. Motor deterioration will be assessed from Part 3 of the UPDRS, but total score will also be reported. ***The assessment will be performed during the "ON" phase, approximately 60 minutes after taking levodopa***.
- Improvement in mood as assessed by the Beck Depression Index (BDI-II): The 21-item BDI–II assesses the intensity of depression. Each item is a list of four statements arranged in increasing severity about a particular symptom of depression.

- Improvement in cognition as assessed by the MMSE: The MMSE is an 11-item examination with a score ranging from 0 – 30 with a higher score indicating better memory. The MMSE will be summarized over time.
- Improvement in sleep as assessed by sleep diaries.
- Change in plasma L-Dopa levels 20, 40, and 60 minutes after L-Dopa and ENT-01 dose: An increase of >30% in peak plasma level during treatment versus baseline would indicate clinically significant increase in absorption attributable to ENT-01 (10 subjects only).
- Change in stool microbiome during treatment: Since ENT-01 has antimicrobial properties in vitro, it may alter the intestinal microbiome; the subject's stool will be analyzed for common intestinal bacteria and viruses at Baseline, Fixed Dose, and Washout periods (10 subjects only).

2.6.4 Pharmacokinetic (PK) Endpoints

PK samples for levodopa will be collected from trial patients according to the instructions detailed in the lab manual and in accordance with the protocol schedule. A separate SAP will be prepared for the PK analysis.

2.6.5 Safety Endpoints

Safety assessments performed during the study include:

- Adverse Events collection and reporting
- Physical Examinations
- Vital sign assessment
- Laboratory assessment including Hematology, Serum chemistry, and Urinalysis
- Electrocardiogram (ECG).
- The Columbia Suicide Severity Rating Scale (C-SSRS)

AEs will be coded according to the latest version of the Medical Dictionary for Regulatory Activities (MedDRA). The intensity/severity of AEs will be graded according to NCI-CTCAE (Common Terminology Criteria for Adverse Events.).

A 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.

The C-SSRS is a questionnaire used to measure of suicidal ideation and behavior.

2.6.6 Tolerability Endpoints

The tolerability endpoint is referred to as the dose limiting toxicity (DLT). These adverse events are considered as DLT:

- Recurrent vomiting defined as 3-5 episodes of vomiting within 24 hours of taking study medication/placebo

- Recurrent diarrhea defined as 4 or more episodes of diarrhea within 24 hours of taking study medication/placebo
- Abdominal pain defined as moderate pain that limits instrumental activities of daily living (ADL) (e.g., preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.) within 24 hours of taking study medication/placebo
- Dizziness defined as lightheadedness or fainting on rising from lying to sitting or standing that limits instrumental ADL and severe enough to indicate non-urgent medical intervention within 24 hours of taking study medication/placebo.

It is anticipated that all subjects receiving active treatment will exhibit a prokinetic effect at a dose lower than DLT and less than or equal to 250 mg. Subjects reaching DLT before a prokinetic effect will be treated at the highest dose at which they did not experience a DLT.

3. SUBJECT POPULATIONS

3.1. Population Definitions

The following subject populations will be evaluated and used for presentation and analysis of the data:

- **Safety Population:** The Safety Population will consist of all subjects who receive at least one dose of study medication during the study. Subjects will be analyzed based on the treatment that they actually received. All safety analyses will be based on this population.
- **Modified ITT Population:** The modified Intent-to-Treat (mITT) Population will include subjects who are randomized and have the stool diary assessments both at baseline and at least one post baseline time-point. Subjects who are found later to have inclusion/exclusion violations will be excluded from the mITT population.
- **Fixed Dose Population:** The Fixed Dose Population will include all subjects who are in mITT Population who enter the Fixed Dose period of the study and are in the treatment period for at least seven days, not including the date of randomization.
- **Efficacy Evaluable Population:** The Efficacy Evaluable Population will include subjects who are in the Fixed Dose Population, complete the Fixed Dose period, are compliant with study medication in the Fixed Dose period, and have no major protocol deviations.
- **Pharmacokinetics Population:** Pharmacokinetics Population will include subjects with a least one L-Dopa PK result.

The precise reasons for excluding subjects from the Efficacy Evaluable Population will be fully defined and documented prior to the unblinded interim analysis.

3.2. Protocol Violations

At the discretion of the sponsor, major protocol violations as determined by a review of the data prior to the database locks or unblinded interim analysis and the conduct of statistical analyses may result in the removal of a subject's data from the Efficacy Evaluable Population. The sponsor, or designee, will be responsible for producing the final protocol violation file (formatted as a Microsoft Excel file), in collaboration with Cytel and the data monitoring group as applicable; this file will include a description of the protocol violation, and clearly identify whether or not this violation warrants exclusion from the Efficacy Evaluable Population. This file will be finalized prior to the database locks.

All protocol violations will be presented in the data listings.

4. STATISTICAL METHODS

4.1. Sample Size Justification

Sample size for this study was determined based on the results from the phase 2a study. In the phase 2a study, efficacy was demonstrated in 34 subjects treated with ENT-01, with a mean change from baseline in CSBMs of 2.4.

For the first part of the study it was anticipated that, out of the 72 randomized subjects, 64 (89%) will be evaluable for the primary efficacy endpoint analysis. Assuming an increase from baseline in weekly CSBM rate in the Fixed Dose period of 2.4 and 0.5 for the ENT-01 and placebo-treated subjects, respectively, and a standard deviation (SD) of 2.0, the study has 90% power to detect a difference between the treatment groups. This power estimate is based on a 2-sided t-test at the 0.05 significance level.

For the second part of the study, enrolling an additional 80 patients in a 1:1 randomization will yield a conditional power of approximately 90%. This is based on the additional subjects having a mean (SD) change from baseline for the Fixed Dose Period of 3.6 (4.11) and 1.3 (2.38) for ENT-01 and placebo, respectively. These parameter estimates are based on the results of the initial cohort of patients in the Phase 2b study. These parameter estimates are based on results of the initial cohort of patients in the Phase 2b study. Eighty additional patients will be randomized to ensure 60 evaluable patients.

4.2. General Statistical Methods and Data Handling

General Methods

All outputs will be incorporated into Microsoft Excel or Word files, sorted and labeled according to the International Conference on Harmonisation (ICH) recommendations, and formatted to the appropriate page size(s).

Tabulations will be produced for appropriate demographic, baseline, efficacy, pharmacokinetic and safety parameters. For categorical variables, summary tabulations of the frequency and percentage within each category (with a category for missing data) of the parameter will be presented. For continuous variables, the mean, median, SD, minimum and maximum values will be presented. Time to event data will be summarized using Kaplan-Meier Methodology using 25^{th}, 50^{th} (median), and 75^{th} percentiles with associated 2-sided 95% confidence intervals, as well as percent of observed event rate and summary statistics for the follow-up time.

Formal statistical hypothesis testing will be performed on the efficacy endpoints with all tests conducted at the 2-sided 0.05 level of significance. Summary statistics will be presented, as well as confidence intervals on selected parameters, as described in the sections below.

For all analyses, baseline will be defined as the most recent measurement prior to the date and time of the administration of ENT-01 or placebo. Therefore, baseline may be on the same day as the dose of study treatment, but prior to the administration of the study treatment.

Computing Environment

All descriptive statistical analyses will be performed using SAS statistical software (Version 9.4), unless otherwise noted. Medical History and Adverse Events will be coding using the latest MedDRA version available upon the study start. Concomitant medications will be coded using the latest World Health Organization (WHO) Drug version available upon the study start.

Methods of Pooling Data

Not applicable to the present study.

Adjustments for Covariates

Statistical analyses for the efficacy endpoints will be adjusted by the randomization strata, the baseline CSBM rate (0 – 0.9 vs. 1.0 – 3.0).

Multiple Comparisons/Multiplicity

Adjustments for multiplicity will not be made for this study.

Subpopulations

The primary and secondary efficacy endpoints will be summarized by the subgroups based on the baseline CSBM rate (i.e., 0 - 0.9 or 1.0 – 3.0). Additionally, the primary and secondary endpoints will be presented by first and second "cohort" (i.e., the 1st cohort of subjects enrolled under the first part via the 3:1 randomization scheme; and the 2nd cohort of subjects randomized via the 1:1 randomization scheme).

Withdrawals, Dropouts, Loss to Follow-up

Subjects who withdrew from the study were not to be replaced.

Missing, Unused, and Spurious Data

When deriving endpoints that use dates, for dates that include missing values, the following conventions will be used.

Value	Imputation
Missing day, month, and year	Not imputed
Missing day and month	Day imputed to June 30th
Missing day	Day imputed to the 15th

When using dates from drug administration pages for the purposes of deriving treatment exposure, the following conventions will apply:

- If the start date is missing, it will be assumed that the first drug intake was given at the date of randomization. This date will replace all first administration missing dates.
- If the last administration date is missing, the date will be imputed with the date taken from the "Status of the Patient at the End of the Treatment" eCRF page (Date of last treatment) or if missing, with the date of last contact taken from the "Status of the Patient" eCRF page.

For missing or partial missing onset dates for adverse events, the following conventions will apply:

The start dates for AEs are important for the following:

1. Treatment-emergent adverse event (TEAE) algorithm. A TEAE is an event that first occurred or worsened in severity after baseline.
2. Designation of unique AE occurrences.
3. Completely missing or partially missing AE onset dates will be imputed after due diligence to obtain accurate AE information has failed. The imputation will consider the start and stop dates relative to the each other, and relative to the date of consent and date of first dose.

AEs with start dates that are completely or partially missing will be analyzed as follows:

- If the start date of an event is completely missing, then the event is assumed to be treatment-emergent.
- If the start date has the month and year but day is missing, the event will be considered treatment-emergent if the month and year of the start date of the event are equal to, or greater than, the month and year of the date of first dose of study drug.

If the start date has the year, but day and month are missing, the event will be considered treatment-emergent if the year of the start date of the event is the same as, or later than, the year of the date of the first dose of study drug.

Visit Windows

No visit windows will be applied.

4.3. Interim Analyses

An unblinded interim analysis will be performed after the last subject completes the Fixed Dose period.

4.4. Subject Disposition

Patient disposition, including analysis population allocation, patients enrolled, completed, and discontinued, and primary reason for discontinuation, will be summarized using frequency and percent. For the purposes of reporting, patients who complete the Fixed Dose period are considered to have completed the trial.

Analysis populations, patients who completed each period (fixed dose, single blind, and washout), and patients who discontinued the study and reasons for discontinuation will be summarized by randomized treatment (placebo or ENT-01). The disposition of subjects in the mITT and safety populations up to the end of the fixed dose period will also be summarized by summarizing the number of subjects who completed the fixed dose period or discontinued study during the fixed dose period, along with the reason for discontinuation. This summary will be done by dose during the fixed dose period. Patients will be counted in every dose that the received during the fixed dose period.

Patients who give informed written consent but are not randomized are considered screen failures. Minimal data, such as demographic information and the reason for screen failure, for patients who fail screening will be recorded on the IXRS. Reasons for screen failure will be summarized.

A by-subject listing of study completion information, including the reason for premature treatment and study discontinuation, if applicable, will be presented.

4.5. Demographic and Baseline Characteristics

Demographics, baseline characteristics and medical history will be summarized for the safety, mITT and fixed dose populations by treatment group (placebo and fixed dose ENT-01) and data listings will be provided as well. Demographic variables, including age, gender, race, ethnicity height, weight, and BMI will be summarized in tabular form. Age (years) will be calculated as (date of informed consent – date of birth +1) / 365.25. Baseline characteristics include age at diagnosis of Parkinson's disease, years with Parkinson's disease, years of constipations, Hoehn and Yahr stage, and constipation severity using baseline CSBM rate.

4.6. Efficacy Analyses

Efficacy analyses to evaluate the treatment effect will be conducted using the Fixed Dose population as the primary population. The analyses will also be performed using the mITT and Efficacy Evaluable populations. Summary statistics will also be presented.

The comparison of active versus placebo treatment will be tested at the two-sided 0.05 significance level.

The date of randomization will not be included for either baseline or post-baseline weekly efficacy endpoints (i.e., rates or mean values). Similarly, the date of the single-blind period visit will also not be used in derivation of weekly efficacy endpoints (rates or mean values). Also, the visit 5 Date (ie, the date the patient begins the Washout period) will be included in the single-blind period (i.e., the single-blind period lasts from the day after Visit 4 up to and including Visit 5).

4.6.1 Derivation of Weekly CSBM and SBM Rates

There are 4 CSBM Definitions, each of which are based on the set of questions from the patient's daily diary. The rates from each of the CSBM definitions will be presented. Note: since the assessments are different between the 2 cohorts the definitions are different.

<u>CSBM Definition 1:</u>

A CSBM is a BM which meets each of the following 3 criteria.

For the 1st Cohort of patients:

1) For this BM the patient responded "No rescue medication used" to the query "Have you used rescue medication within 24 hours prior to this bowel movement?"
2) For this BM the patient responded
   - "Yes" to the query "Did you feel like you completely emptied your bowels?"

   OR

   - "No" to the query "Did you feel like you completely emptied your bowels?" but the BSFS Ease of Passage score is 6 or 7.
3) The bowel movement has a BSFS Ease of Passage score of 3 or greater (or missing).

For the 2nd Cohort of patient:

1) No rescue mediation was taken within 24 hours of the Bowel Movement (note: the date and time of the bowel movements and rescue medication (i.e., laxative) use are included in the subjects daily diary data).
2) For this BM the patient responded :
   - "No" to the query "Do you feel like you have more poop left to pass?"

   OR

   - "Yes" to the query "Do you feel like you have more poop left to pass?" and the BSFS Ease of Passage score is 6 or 7.
3) The bowel movement has a BSFS Ease of Passage score of 3 or greater (or missing).

CSBM Definition 2:

The 2nd CSBM definition removes the BSFS criteria from the CSBM definition. A CSBM is a BM which meets each of the following 2 criteria

For the 1st Cohort of patients:

1) For this BM the patient responded "No rescue medication used" to the query "Have you used rescue medication within 24 hours prior to this bowel movement?"
2) For this BM the patient responded "Yes" to the query "Did you feel like you completely emptied your bowels?"

For the 2nd Cohort of patient:

1) No rescue mediation was taken within 24 hours of the Bowel Movement (note: the date and time of the bowel movements and rescue medication (i.e., laxative) use are included in the subjects daily diary data).
2) For this BM the patient responded "No" to the query "Do you feel like you have more poop left to pass?"

CSBM Definition 3:

The 3rd CSBM definition broadens the definition of a CSBM for the 2nd cohort. A CSBM is a BM which meets each of the following 2 criteria

For the 1st Cohort of patients:

1) For this BM the patient responded "No rescue medication used" to the query "Have you used rescue medication within 24 hours prior to this bowel movement?"
2) For this BM the patient responded "Yes" to the query "Did you feel like you completely emptied your bowels?"

For the 2nd Cohort of patient:

1) No rescue mediation was taken within 24 hours of the Bowel Movement (note: the date and time of the bowel movements and rescue medication (i.e., laxative) use are included in the subjects daily diary data).
2) For this BM the patient responded
   - "No" to the query "Do you feel like you have more poop left to pass?"

     OR
   - "Yes" to the query "Are you Done for the Day"

     OR
   - "Yes" to the query "Did you feel like you completely emptied your bowels?"

CSBM Definition 4:

This is a more stringent definition for CSBMs for Cohort 2, requiring each of the 3 completeness questions to be appropriately answered. A CSBM is a BM which meets each of the following 3 criteria.

For the 1st Cohort of patients:

1) For this BM the patient responded "No rescue medication used" to the query "Have you used rescue medication within 24 hours prior to this bowel movement?"
2) For this BM the patient responded
   - "Yes" to the query "Did you feel like you completely emptied your bowels?"

   OR
   - "No" to the query "Did you feel like you completely emptied your bowels?" but the BSFS Ease of Passage score is 6 or 7.
3) The bowel movement has a BSFS Ease of Passage score of 3 or greater (or missing).

For the 2nd Cohort of patient:

1) No rescue mediation was taken within 24 hours of the Bowel Movement (note: the date and time of the bowel movements and rescue medication (i.e., laxative) use are included in the subjects daily diary data).
2) For this BM the patient met either of these 2 criteria:

   2a) The patient responded:
   - "No" to the query "Do you feel like you have more poop left to pass?"

     AND
   - "Yes" to the query "Are you Done for the Day"

     AND
   - "Yes" to the query "Did you feel like you completely emptied your bowels?"

   OR

   2b) The BSFS Ease of Passage score is 6 or 7.

3) The bowel movement has a BSFS Ease of Passage score of 3 or greater (or missing).

An SBM is a BM which meets the following criteria:

- For this BM the patient responded "No rescue medication used" to the query "Have you used rescue medication within 24 hours prior to this bowel movement"

A patient's CSBM weekly rate over a particular period will be derived using the following algorithm:

Weekly CSBM Rate = 7×{# of CSBMs in the period}/{# of days in the period}

The weekly SBM rate calculations use the same algorithm.

If a patient is missing BM information for a day, it will be assumed that the patient had 0 SBMs and 0 CSBMs for that period (with the exceptions in calculating Baseline CSBM and SBM rates noted in Section 2.6).

If a subject diary has "repeat" stool data (eg, a BM repeated with the same date/time of BM) only the latest duplicate observation relative to when the patient entered the data into the diary will be used.

For the calculation of the Baseline CSBM (SBM) weekly rate:

- If the patient has more than 14 days of diary data during the Screening Period, only the last 14 days with non-missing diary entries prior to the randomization visit will be used (i.e., the days which are more than 14 days prior to the randomization visit will be excluded)
- If a patient has 11- 14 days of diary data, all available diary days will be used.
- If a subject has more than two weeks of entries during the screening period, consecutive missing entries during the screening period will be excluded from baseline calculations, and the last fourteen days with entries during the screening period will be used to derive baseline weekly rates.

For analyses of the CSBM (SBM) in the Fixed dose period, if a patient has not been in the Fixed Dose period for at least seven days, then the number of days used for calculating the weekly CSBM (SBM) rate will be adjusted to seven days by using the last "n" days of the Dose Escalation period leading up to the fixed dose period to get to 7 days in the Fixed Dose period. This derivation will be used for all efficacy endpoints for the Fixed Dose Period (e.g., stool ease of passage and stool consistency).

For analyses of the CSBM (SBM) rate during the single-blind placebo and washout periods, all available data in those periods, excluding observations on the date of the single-blind placebo visit as described before, will be used in the derivations of weekly rates described at the beginning of this section.

4.6.2 Stool Consistency and Ease of Passage Analysis Conventions

Only stool consistency and ease of passage scores associated with SBMs will be used in analyses (i.e., excluding BMs where it was indicated that rescue medication had been used within 24 hours).

4.6.3 Analysis of the Primary Efficacy Endpoint

The primary efficacy endpoint is the change from baseline in weekly CSBM rate during the Fixed Dose period. The primary analysis will be based on the Fixed Dose Population. The primary efficacy analysis will be performed via an analysis of covariance (ANCOVA) model with change from baseline in weekly CSBM rate as the dependent variable, treatment (ENT-01 or placebo) as the independent variable, and baseline weekly CSBM rate as a covariate.

As a supplemental analysis, the treatment effect for the primary efficacy endpoint will be summarized by the weekly CSBM rate subgroups (0 – 0.9, 1.0 – 3.0). The analysis will be performed for both Efficacy Evaluable population and Fixed Dose population.

The study will be considered a success if the p-value for the comparison of treatment groups from the ANCOVA model using the Fixed Dose population is significant ($p<0.05$).

Change from baseline in weekly CSBM rate will also be summarized by the specific fixed dose for patients. These summaries will be descriptive in nature only and no inferential statistics will be performed.

A linear trend test for weekly CSBM rate within each treatment group across baseline, fixed dose, and single blind periods will be conducted by using mixed model repeated measures (MMRM) methods, where the Baseline, Fixed Dose, and Single Blind periods are assigned values of 0,1, and 2 respectively for modeling purposes. Baseline CSBM rate (0-0.9/1.0-3.0) is included as a factor, with subject intercept and slope as random effects in the mixed model. The trend test will also be conducted across all subjects, including both Placebo and ENT-01 treatment groups in the model as a factor (fixed effect).

4.6.4 Analysis of Secondary Efficacy Endpoints

All secondary efficacy endpoints will be analyzed based on both the Efficacy Evaluable population and Fixed Dose population.

Continuous Secondary Efficacy Endpoints

The following are the set of continuous secondary efficacy endpoints:

- Change from baseline in the weekly SBM rate during the Fixed Dose period
- Change from baseline in stool ease of passage during the Fixed Dose period
- Change from baseline in stool consistency during the Fixed Dose period
- Change from baseline in suppository/enema use during the Fixed Dose period
- Change from baseline in the overall scores and individual items of PAC-SYM and PAC-QOL subjective assessments during the Fixed Dose period

All continuous secondary efficacy endpoints will be analyzed in the same manner as the primary efficacy endpoint with treatment, baseline CSBM strata, and corresponding baselines as the independent variables in the ANCOVA model and also by summarizing change from baseline

by the specific fixed doses. The same linear trend tests will also be performed on these endpoints.

Categorical Secondary Efficacy Endpoints

The following are the set of categorical secondary efficacy endpoints:

- Dichotomous prokinetic bowel response during the Fixed Dose period
- Dichotomous SBM bowel response during the Fixed Dose period
- Frequency of dose adjustments in the Dose Adjustment period from the initial starting dose to the subject's fixed dose

The dichotomous endpoints will be summarizes using count data, frequencies, and odds ratios for comparisons between groups, and analyzed using Fisher's exact test. If there are sufficient numbers in each strata, the analysis may also be performed using the Cochran-Mantel-Haenszel (CMH) test adjusting for the baseline CSBM strata, to compare ENT-01 versus placebo. The frequency of dose adjustment will be summarized by treatment groups and baseline CSBM strata and no formal test will be performed to compare the treatment groups.

Summary statistics will also be presented by specific fixed dose groups for descriptive purposes only.

Time to Event Secondary Efficacy Endpoints

The following are the set of time to event secondary efficacy endpoints:

- Time to first CSBM in the Fixed Dose period
- Time to first SBM in the Fixed Dose period
- Time to first rescue medication use in the Fixed Dose period

Time to event endpoints will be summarized using Kaplan-Meier methods and comparisons between treatment groups will be made via log-rank tests, stratified by the baseline CSBM rate. The start date for time to event analyses will be the date of first dose in the fixed dose period.

4.6.5 Analysis of Exploratory Efficacy Endpoints

Continuous Exploratory Efficacy Endpoints

Continuous endpoints include change from baseline of the following outcomes:

- Weekly CSBM rate during the Placebo Withdrawal and Washout periods
- Weekly SBM rate during the Placebo Withdrawal and Washout periods
- Stool ease of passage during the Placebo Withdrawal and Washout periods
- Stool consistency during the Placebo Withdrawal and Washout periods
- Suppository/enema use during Placebo Withdrawal and Washout periods
- Total score and 3 subdomain scores from PAC-SYM during the Placebo Withdrawal and Washout periods
- Total Score and 4 subdomain scores from PAC-QoL during the Placebo Withdrawal and Washout periods
- Stool microbiome during each study period, defined as the number of species found in the microbiome

- Total sleep time (hours) per day during each study period
- Delay (minutes) in sleep onset per night during each study period
- Total awake time (hours) per night during each study period
- Number of wakes per night during each study period
- Total score from UPDRS and individual part scores to assess the symptoms of Parkinson's Disease during each study period
- Total score from BDI-II to assesses the intensity of depression during each study period
- Total score from MMSE to assess cognition during each study period

All continuous exploratory efficacy endpoints will be analyzed in the same manner as the primary efficacy endpoint with treatment, baseline CSBM strata, and corresponding baselines as the independent variables in the ANCOVA model. Changes to the single blind placebo and washout periods will also be repeated using the fixed dose rate or end of fixed dose measurement as baseline. Summary statistics will also be presented by specific fixed dose groups for descriptive purposes only.

Categorical Exploratory Efficacy Endpoints

The following is the lone categorical exploratory efficacy endpoint:

- Improvement in frequency and/or severity of hallucinations/delusions as measured by SAPS-PD score during the Fixed Dose period over baseline for subjects with SAPS-PD score >0 at the Screening visit. Improvement is defined as ≥2.33 points reduction in score from baseline on the SAPS-PD

The endpoint will be analyzed using Fisher's exact test, and also by the Cochran-Mantel-Haenszel (CMH) test adjusting the baseline CSBM strata, if numbers permit, to compare ENT-01 versus placebo. Summary statistics will also be presented by specific fixed dose groups for descriptive purposes only.

4.7. Pharmacokinetic Analysis

The plasma concentration data and PK parameters of L-Dopa will be summarized based on the PK population by time point (pre-dose, 20, 40 and 60 minutes post-dose) and dose level. These measures will be presented in listings as well.

An increase of >30% in peak plasma level of L-Dopa during treatment from baseline would indicate clinically significant increase in absorption attributable to ENT-01. The frequency and percentage of subject with clinically significant increase will be tabulated without formal statistical analysis for the PK population.

4.8. Safety Analyses

All safety analyses will be conducted on the Safety Population. The safety data will be presented in individual listings and summary tables. Descriptive statistics will be used to summarize the safety data by period and both by treatment group and overall. The denominator for calculating the percentages will be based on the number of subjects on each treatment arm (ENT-01 or placebo) and overall in the Safety Population. The summary for the ENT-01 arm during the Fixed Dose period will also be broken down to the fixed dose levels.

No formal hypothesis-testing analysis of safety data will be performed.

4.8.1 Extent of Exposure

The extent of exposure will be summarized by the number of tablets received and number of days on treatment during the Dose Adjustment period and Fixed Dose period.

4.8.2 Adverse Events

Adverse events (AEs) will be coded by system organ class (SOC) and preferred term (PT) according to the latest version of the Medical Dictionary for Regulatory Activities (MedDRA). The intensity/severity of AEs will be graded according to the latest NCI-CTCAE. Treatment-emergent AEs (TEAEs) are defined as any AE occurring or worsening on or after the first dose of study treatment. AEs that have a possible, probable or definite relationship to study drug will be defined to be related to the study drug while others will be defined as “not related”. AEs with the closest relationship to the study drug will be used for summary.

The following types of summaries will be provided:

- Overview of TEAEs
- TEAEs by SOC and PT
- TEAEs by SOC, PT, and Maximum CTCAE toxicity grade
- TEAEs by SOC, PT, and Maximum CTCAE toxicity grade >= 3
- TEAEs by SOC, PT, and closest relationship to study drug
- TEAEs by PT in decreasing frequency
- Drug-related TEAEs by SOC and PT
- Drug-related TEAEs by PT in decreasing frequency
- Serious TEAEs by SOC and PT
- TEAEs leading to study treatment discontinuation by SOC and PT
- TEAEs leading to dose interruption by SOC and PT

All adverse event summaries will be presented by the dose the subject was on at the time of the AE. Adverse event summaries will also be presented during the following time periods:

- Dose Escalation
- Fixed Dose
- Dose Escalation and Fixed Dose (Treatment Period)
- Single Blind Placebo

- Washout
- Entire Study

In these tabulations, if a subject experiences the same preferred term multiple times within a period, then the event will be counted only once within the period.

In tabulation by severity (i.e., CTCAE toxicity grade),

- For a given preferred term, only the most severe preferred term for each subject will be included.
- For a given system organ class, only the most severe system organ class for each subject will be included.

Similarly, in tabulation by relationship,

- For a given preferred term, the most closely related preferred term to the study drug for each subject will be included.
- For a given system organ class, the most closely related system organ class to the study drug for each subject will be included.

TEAEs, serious TEAEs, and AEs leading to discontinuation from the study treatment will be provided in separate data listings.

4.8.3 Deaths

All deaths and reasons for death will be provided in a listing.

4.8.4 Physical Examinations

Summaries of physical examinations will present frequency distribution of abnormal findings by body system and visit. The denominators for calculating the percentages will be based on the number of subjects evaluated for a particular body system. All physical examination findings will be presented in a data listing.

4.8.5 Laboratory Data

Clinical labs will be performed by a central laboratory. Labs to be drawn during the study include serum chemistries, a hematology panel, coagulation panel and urine analysis. Clinical laboratory values will be expressed using conventional units.

Descriptive statistics by period (or visit) and treatment group will be presented for each laboratory parameter. Changes from Baseline as well as shift tables will be presented. All laboratory values will be classified as normal, below normal, or above normal based on normal ranges supplied by the central laboratory. Frequencies of abnormal values and frequencies of clinically significant abnormal values will be presented in tabular form. In the event of repeat values, the last non-missing value per study period will be used.

All laboratory data will be provided in data listings.

4.8.6 Vital Signs

The actual value and change from baseline to each on study evaluation will be summarized for vital signs. By-subject listings of vital sign measurements will be presented in data listings.

4.8.7 Electrocardiogram

ECG results will be summarized descriptively, including the number and percent of subjects with normal, abnormal and clinically significant abnormal results at Baseline and each study visit. The denominators for calculating the percentages will be based on the number of subjects with non-missing values in each assessment.

All ECG data for each subject will be provided in data listings.

4.8.8 Columbia-Suicide Severity Rating Scale (C-SSRS)

The number and percentage of patients with Suicidal Ideation, Suicidal Behavior, and Self-Injurious Behavior without Suicidal Intent based on the Columbia-Suicide Severity Rating Scale (C-SSRS) during the treatment period will be summarized by treatment group. Treatment-emergent Suicidal Ideation, Suicidal Behavior, and Self-Injurious Behavior without Suicidal Intent will be based on Visit 3 through End of Study (EOS)/Early Termination (ET). In this summary, the number and percentage of patients who experience the particular event at least once during the treatment period will be summarized. The particular events are:

- Suicidal Ideation (1-5)
    1. Wish to be Dead
    2. Non-specific active suicidal thoughts
    3. Active suicidal ideation with any methods (not plan) without intent to act
    4. Active suicidal ideation with some intent to act, without specific plan
    5. Active suicidal ideation with specific plan and intent
- Suicidal Behavior (6-10)
    6. Preparatory acts or behavior
    7. Aborted attempt
    8. Interrupted attempt
    9. Non-fatal suicide attempt
    10. Completed suicide
- Self-injurious behavior without suicidal intent

For the composite endpoint of suicidal ideation (Categories 1-5), the number and percentage of patients who experience any one of the five suicidal events at least once during the treatment period will be summarized by treatment group. For the composite endpoint of suicidal behavior (Categories 6-10), the number and percentage of patients who experience any one of the five

suicidal behavior events at least once during the treatment period will be summarized by treatment group.

Two shift tabulations from baseline for the C-SSRS will be produced by treatment group during the treatment period. The Baseline C-SSRS assessment includes both (A) Lifetime Assessment (for both the suicidal behavior and for the suicidal ideation sections) and (B) Recent Assessment: 1 year prior to study start for the suicidal behavior section, and 1 month prior to the study start for the suicidal ideation section. A separate shift tabulation will be produced using each of these two baselines. The three groupings for the shift tables are: (a) No suicidal ideation or behavior, (b) Suicidal Ideation, and (c) Suicidal Behavior. Suicidal Ideation includes any one of the five suicidal ideation events (Categories 1-5). Suicidal behavior includes any one of the five suicidal behavior categories (Categories 6-10). Each patient is counted in one cell only for each of the two tabulations. Patients with both Suicidal Ideation and Suicidal Behavior are included in the Suicidal Behavior category for the particular tabulation.

Patient listings for the C-SSRS will also be produced.

4.8.9 Concomitant Medications

Prior medications are defined as medications taken prior to the first dose of study drug (ENT-01 or placebo). Concomitant medications are defined as medications taken on or after the first dose study drug.

Prior and Concomitant medications will be coded using the WHO Drug dictionary. Results will be tabulated by Anatomic Therapeutic Class (ATC) and preferred term.

The use of prior and concomitant medications will be included in by-subject data listing.

4.8.10 Tolerability

Tolerability will be measured by DLT endpoints. The frequency of occurrence for each tolerability endpoint including recurrent vomiting, recurrent diarrhea, abdominal pain and dizziness will be summarized by treatment group and dose level for the Dose Adjustment period. Percentages will be based on the number of subjects in each dose level in the Safety Population. Since this occurs during the dose adjustment period, the dose level is not fixed for each patient. The number of patients in a dose level will be calculated based on the highest dose level a patient ever took during the Dose Adjustment Period. For example if a patient with low baseline CSBMs had the highest dose of 200 mg, then this patient would be included in the doses of 150, 175, and 200 mg.

5. CHANGES TO PLANNED ANALYSES

As of this date, there have been no changes between the protocol-defined statistical analyses and those presented in this statistical plan.

6. REFERENCES

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