STATISTICAL ANALYSIS PLAN: ENT-01

Study Title:	**A Multicenter, Single-Dose, Multiple-Dose, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Orally Administered ENT-01 for the Treatment of Parkinson's Disease Related Constipation**
Protocol Number:	ENT-01
Protocol Version/ Date:	27JUL2017 and 02FEB2018
Study Phase:	1/2a
Product Name:	ENT-01
Sponsor:	Enterin Inc.
Sponsor Contact:	2005 Market Street, Suite 3125 Philadelphia, PA 19103

	Date
Statistical Analysis Plan Date:	04May2018
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SPONSOR SIGNATURE PAGE

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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 guidance and guidelines.

Author

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Director of Biostatistics Date:

LLX Solutions, LLC

Approver

Denise Barbut, MD FRCP Signature:

Date:

President and CMO

Enterin Inc.

TABLE OF CONTENTS

TABLES INCLUDED IN THE TEXT

ABBREVIATIONS

Abbreviation	Definition of Terms
AE	Adverse Events
AUC_{0-24}	Area under the concentration versus time curve from time 0 to the concentration at 24 hours; calculated using linear trapezoid rule
BDI	Beck Depression Inventory
BLQ	Below the Quantification Limit
BMI	Body mass index
CL/F	Clearance defined as: Dose/AUC_{0-24}
C_{max}	Maximum or peak measured plasma concentration
C_{min}	Minimum or trough measured plasma concentration
CSR	Clinical Study Report
CV	Coefficient of Variation
DLT	Dose limiting Toxicity
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FAS	Full analysis set
LFT	Liver Function Test
LSTDEi	Consistently lowest clinically effective safe and tolerable dose for that particular individual
MCT	Mean Colonic Transit
MDRP	Medical Data Review Plan
MedDRA	Medical Dictionary for Regulatory Activities
MMSE	Mini Mental State Examination
NC	Not Calculated

Abbreviation	Definition of Terms
NMSQ	Non-Motor Symptoms Questionnaire
PAC-QoL	Patient Assessment of Constipation Quality of Life
PAC-SYM	Patient Assessment of Constipation Symptoms
PD	Pharmacodynamics
PDSS	Parkinson's Disease Sleep Scale
PFS-16	Parkinson's Disease Fatigue Scale
PK	Pharmacokinetics
PT	Preferred Term
Q1	First Quartile
Q3	Third Quartile
RBD	REM behavior disorder
RBDQ	Rem Sleep Behavior Disorder Screening Questionnaire
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
STDE	Safe and Tolerable dose in all and pro-kinetic Effect in at least some subjects
SOC	System Organ Class
$t_{1/2}$	Apparent terminal elimination half-life; calculated as $\ln(2)/ \lambda_z$. 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.
TEAEs	Treatment Emergent Adverse Events
T_{max}	Time of maximum or peak measured plasma concentration
ULN	Upper limit of normal

Abbreviation	Definition of Terms
UM-PDHQ	The University of Miami Parkinson's Disease Hallucinations Questionnaire
UPDRS	Unified Parkinson's Disease Rating Scale
WHO	World Health Organization
WHO-DD	World Health Organization Drug Dictionary
λ_z	Apparent terminal rate constant

1 INTRODUCTION

This statistical analysis plan (SAP) is designed to outline the statistical methods to be used for analyses and data presentation for reporting pharmacokinetics (PK), pharmacodynamics (PD), safety and tolerability, and any exploratory objectives for study protocol ENT-01. This document has been prepared based on Study Protocol dated 27 July 2017 and Protocol amendment 02 February 2018.

This SAP supersedes the statistical considerations identified in the protocol. If additional analyses are required to supplement the planned analyses described in this SAP, they may be performed and will be identified in the CSR. Any substantial deviations from this SAP will be agreed upon between Enterin Inc. and LLX Solutions, LLC and identified in the CSR.

This SAP amendment was done for

1. Adding details on the scoring methods for each questionnaire to be analyzed in the study;
2. Deleting two secondary analyses for "Success" endpoint:
    - Due to the protocol amendment, the sample size for randomization period became too small to do statistical analysis. Therefore, it was deleted from this SAP amendment.
    - During the trial, it became apparent that the period for "Success" at the end of the first week in washout period is not consistent with the washout period used for other pharmacodynamics parameters. Therefore, this analysis is also deleted in the SAP amendment.
3. Clarifying the summaries and analyses should be provided for fixed dosing or fixed doing period as applicable.

2 STUDY DESIGN OVERVIEW

2.1 Overall Study Design

This is a single-dose, multiple-dose, randomized, double-blinded, placebo-controlled, two-staged study in Parkinson's disease subjects with constipation. The study will consist of two stages, each with run-in period and dosing periods and followed by a wash-out period of 2 weeks. Stage 1 will consist of 10 subjects while Stage 2 will consist of 40 subjects to ensure at least 34 evaluable subjects are obtained. During Stage 1, after providing informed consent, subjects will be screened for eligibility. Qualified subjects will proceed through all the dose escalations of ENT-01 starting at 25 mg and ending at 200 mg or at the dose at which s/he experiences a dose limiting toxicity (DLT). Stage 1 is staggered, where the first two subjects will be administered a single dose of 25 mg first. The rest of the subjects will be dosed after the first two subjects have been observed for 72 hours with no safety concerns. The doses to be tested are 25mg, 50mg, 75mg, 100mg, 125mg, 150mg, 175mg and 200mg. Subjects will then be observed off study drug for two weeks during the wash-out period. STDE (Safe and Tolerable dose in all and pro-kinetic Effect in at least some) dose will be identified and carried over to Stage 2.

In Stage 2 under protocol dated 27 July 2017, subjects who signed the informed consent and are eligible will go through a multiple dose escalation (3 dosing days at each dose) period. The dose range is 75 mg up to 175 mg with an increment of 25 mg for next higher dose. Ten subjects will be in the sentinel group and will be dosed first. The dose at which the subject experiences a consistent pro-kinetic effect (spontaneous, complete stool in at least 2/3 consecutive doses) without a DLT will be the dose selected going forward for that subject. This will be the LSTDEi (i.e. consistently clinically effective safe and tolerable dose for that particular individual). After the LSTDEi is established, subjects will continue to take this dose for 2-4 days, followed by randomization on the LSTDEi dose with a 1:1 ratio to ENT-01 or placebo in block sizes of 4 for a 6-day period (period 4). Seven subjects from the ENT-01 group and three subjects from the control group will be in the PK group and have the PK measurement taken. The rest will be in the non-PK group. All the subjects will then enter the wash-out period for two weeks.

The protocol dated 27 July 2017 was amended during the trial. And the protocol amendment was finalized on 02 February 2018. In this amendment, Stage 2 dose range is from 100 mg to 250 mg with an increment 25 mg for next higher dose. It also removed randomization period.

Except for the above changes, there were no other changes from protocol dated 27 July 2017

Table 1: Overall Study Design

STAGE 1			STAGE 2A			
Period 1	Period 2.1	Period 2.2	Period 3.1	Period 3.2	Period 4	Period 5
Run-in sentinel group (Cohort 1)	Single dose escalation sentinel group	Washout	Run-in (Cohort 2)	Identification of prokinetic dose using a 10 subject sentinel group followed by the remaining subjects	1:1 Randomization onto placebo or prokinetic dose	Washout
2 weeks N=10	3 weeks N=10	2 weeks N=10	2 weeks N=40	≤17days N=40	6 days N=40	2 weeks N=40

Table 2: Schedule of Assessments of Stage 1

Period	**1 Run-In Sentinel 2 wks**		**2.1 Single Dose 3-8 wks**	**2.2 Wash-Out 2 wks**
	D1	**D14**	**D1**	
Consent	X			
Demographics	X			
Inclusion/exclusion	X	X		
Medical history	X			
Physical exam	X		X	X
Vital signs[1]	X		X	X
Rome-IV	X			
Blood chemistry	X		X	X
Hematology	X			
Urinalysis	X			X
Fecal occult blood	X		X	X
EKG	X		X	X
Pregnancy test	X			
Monitoring			X	X
Discontinue medications	X			
Re-start medications				X
Diary entries	X	X	X	X
UPDRS	X		X[4]	X[2]
I-Button[3]	X	X	X	
PAC-QOL	X	X	X[4]	X[2]
PAC_SYM	X	X	X[4]	X[2]
RBDQ	X		X[4]	X[2]
NMSQ	X		X[4]	X[2]
PFS-16	X		X[4]	X[2]
UM-PDHQ	X		X[4]	X[2]
BDI	X		X[4]	X[2]
Trail-making A and B	X		X[4]	X[2]

Table 2: Schedule of Assessment of Stage 1

Period	**1 Run-In Sentinel 2wks**		**2.1 Single Dose 3-8 wks**	**2.2 Wash-Out 2 wks**
	D1	**D14**	**D1**	
MMSE	X		X[4]	X[2]
Adverse events		X	X	X
PK samples			X[5]	
Schedule visit days	X			
Administer ENT Dose			X	
Record Medications	X	X	X	X
Daily phone calls to on non-visit days		X	X	X

[1]Vital signs taken before dose and 2 hours after dose

[2]End of period 2

[3]I-Button placed days 9 of run-in periods and worn continuously through day 5 of wash-out periods.

[4]Surveys taken at last dosing visit

[5]PK samples taken pre-dose and 1, 2, 4, 8 and 24 hours post-dose

Table 3: Schedule of Assessments of Stage 2 under Protocol Dated 27 July 2017

Scheduled Assessments	Period 3.1 Run-In					Period 3.2 Multiple Dose		Period 4 Randomized Treatment			Period 5 Wash-Out	
	Day 1 Visit 1	Days 2-7, 9-10, 12-13 Calls	Day8 Call	Day 11 Visit 2	Day l4 Call	Days. 1-13, 15-16 Calls	Day l4 Call	Day 1 Visit 3[6]	Days 2-5 Calls	Day6 Visit 4	Days 1-7 Calls	Day l4 Visit 5
Informed Consent	X											
Inclusion/Exclusion Criteria	X											
Demographics/Medical History	X											
Prior and Con Meds	X											
Complete Physical Exam	X											X
Height	X											
Weight	X			X				X		X		X
Vital Signs[1]	X			X				X		X		X
ECG	X							X				X
Local Labs -Chemistry, Hematology	X							X		X		X
Local Labs - Urinalysis, Serum Pregnancy	X											
Fecal Occult Blood	X							X				X
Randomization								X				
Study Drug Dispense, Accountability, or Collection				X				X		X		
Reminder to Begin Taking Study Drug the Next Morning					X							
Instructions to Discontinue Laxatives and other BM Medication	X											
Instructions to Discontinue Laxatives and other BM medications												X
Stool and Sleep eDiary Instruction/Review	X	X	X	X	X	X	X	X	X	X	X	X
AE Review		X	X	X	X	X	X	X	X	X	X	X
Rome IV questionnaire	X		X									
UPDRS	X							X				X
PAC-QOL and PAC-SYM	X				X		X	X		X		X
Questionnaires- BDI, NMSQ, PFS-16, UM-PDHQ, MMSE, Trail-Making A and B, RBDQ and PDSS	X							X		X		X
Sitzmarks Capsule Distributions[2]	X							X				
Reminder to Take Sitzmarks Capsules[3]		X							X			
Abdominal X-Ray				X						X		
Dispense I-Button[4]	X											

Scheduled Assessments	Period 3.1 Run-In					Period 3.2 Multiple Dose		Period 4 Randomized Treatment			Period 5 Wash-Out	
	Day 1 Visit 1	Days 2-7, 9-10, 12-13 Calls	Day8 Call	Day 11 Visit 2	Day l4 Call	Days. 1-13, 15-16 Calls	Day l4 Call	Day 1 Visit 3[6]	Days 2-5 Calls	Day6 Visit 4	Days 1-7 Calls	Day l4 Visit 5
PK Draws & LFT[5]								X		X		
Phone Call		X	X		X	X	X		X		X	

[1] For PK patients, vital signs taken pre-dose and 2 hours after dose. Blood pressure will be taken for all patients twice, first lying down and then after 5 minutes of sitting or standing from the recumbent position. All other vital signs will be measured after the patient has been resting in a sitting position for at least 5 minutes.

[2]Sitzmark capsules are dispensed to patients at Visit 1 with instructions to take one a day on Days 8, 9, and 10 of the Run-In period for the abdominal X-ray on Day 11. Patients will receive more Sitzmark capsules at Visit 3 with instructions to take one a day on Days 3, 4, and 5 of the Randomized Treatment period for the abdominal X-ray on Day 6.

[3]Subjects are reminded during the Day 7 call of the Run-In period and Day 2 call of the Randomized Treatment period to begin taking the Sitzmark capsules the following day to prepare for the abdominal X-ray on Day 11 of the Run-In period and Day 6 of the Randomized Treatment period.

[4]Patients will wear an I-Button continuously from Day 8 of the Run-In period to Day 5 of the Wash-Out period except during periods of bathing.

[5]PK samples are taken pre-dose and 1, 2, 4, and 8 hours post-dose. A sample for liver function tests will be taken with the 8-hour post-dose PK draw.

[6]Visit 3 occurs on the day before randomization in non-PK subject

Table 4: Schedule of Events of Stage 2 under Protocol Dated 02 February 2018

Scheduled Assessments	Period 3.1 Run In					Period 3.2 Dose Escalation		Period 4 Fixed Dos		Period 5 Wash Out		
	Day 1 Visit 1 Screening	Days 2-7, 9,10,12,13 Phone Calls	Day 8 Phone Call	Day 11 Visit 2	Day 14 Phone Call	Days 1-13 Phone Calls	Day 14 Phone Call	Days 1-3 Phone Calls	Day 4 Visit 3	Days 1-7 Phone Calls	Day 14 Visit 4	Day 28 FU Call
Informed Consent	X											
Inclusion/Exclusion Criteria	X											
Demographics/Medical History	X											
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X
Complete Physical Exam	X										X	
Height	X											
Weight	X			X					X		X	
Vital Signs (1)	X			X					X		X	
EKG	X								X		X	
Local Labs: Chemistry*, Hematology*	X								X		X	
Local Labs: Coagulation Panel*	X											
Local Labs: Serum Pregnancy (2a) or FSH (2b)	X											
Urinalysis* (dipstick)	X								X		X	
Urine Pregnancy Test (3)				X					X		X	
Fecal Occult Blood (4)	X								X		X	
Study Drug Dispense, Accountability, Collection				X					X			
Reminder to Begin Taking Study Drug the Next					X							
Instruction to Discontinue Laxatives and Other Restricted Medications (5)	X											
Instruction to Resume Laxatives and Other Restricted Medications										X (6)		
Stool and Sleep Diary Instruction/Review	X	X	X	X	X	X	X	X	X	X	X	
AE Assessment and Review	X	X	X	X	X	X	X	X	X	X	X	X
Rome IV Questionnaire	X		X									
UPDRS	X								X		X	
PAC-QOL and PAC-SYM	X				X		X		X		X	
BDI-II, NMSQ, PFS-16, UM-PDHQ, MMSE, Trail-Making A and B, RBDQ, PDSS	X								X		X	
I-Button	X (7)		X							X (9)	X (10)	

* Refer to Appendix 1 for full list of clinical laboratory assessments

(1) The first blood pressure and heart rate will be taken after the subject has been in the supine position for at least 5 minutes. The second blood pressure and heart rate reading will be taken while the subject is standing, within 5 minutes of standing from the supine position. All other vital signs (respiration rate and body temperature) will be taken after the subject has been sitting or standing for 5 minutes.

(2) (2a) For women of child-bearing potential and (2b) For post-menopausal women less than 60 years of age.

(3) For women of child-bearing potential, done on site with urine pregnancy kit provided

(4) Subjects will be provided a home testing kit with instructions; to be used at home on the first bowel movement following the visit, and results to be given to the study coordinator at subsequent phone call visit.

(5) Subjects will be instructed to discontinue all laxatives, bulking agents, softeners and suppositories as well as clonazepam, opiates, etc. as specified in the protocol. Proton pump inhibitors and antacids may be continued until the end of the Run-In Period.

(6) Subjects will be instructed to resume use of their regular regimen laxatives, bulking agents, softeners, proton pump inhibitors, and antacids after week 1 of wash out.

(7) Dispense I-Button

(8) Instruct subjects to begin wearing the I-Button

(9) Instruct subjects to stop wearing the I-Button at the end of Day 5 of Wash Out

(10) Collect I Button

2.2 Sample Size/ Power Calculation

No formal sample size calculation was performed for Stage 1. The number of subjects is based on feasibility and is considered sufficient to meet the objectives of the study.

For Stage 2, assuming the highest proportion of spontaneous resolution of constipation with no treatment to be 0.10, 34 evaluable subjects who have measurements at both baseline and at the end of the fixed dose period will provide 80% power to detect the difference between 0.10 (proportion expected if patients are not treated), p0, and an ENT-01 proportion, pE, of 0.29. Assuming a drop-out rate of 15%, a total of 40 subjects will ensure at least 34 evaluable subjects will be obtained.

2.3 Randomization and Blinding

No randomization is performed for Stage 1.

During randomization period of Stage 2 under the protocol dated 27 July 2017, subjects will be randomly allocated in equal proportion (1:1) to 1 of 2 double-blind treatment groups:

- ENT-01 – Patients will remain at dose level taken in period 3.2 of Stage 2
- Placebo – Patients will be switched to the same number of placebo tablets as the dose level taken in period 3.2 of Stage 2

3 STUDY OBJECTIVES

3.1 Objectives for Stage 1

3.1.1 Primary Objective

To investigate the safety and tolerability of single oral doses of ENT-01 in patients with Parkinson's disease related constipation.

3.1.2 Secondary Objective

To evaluate the pharmacokinetic (PK) characteristics of single oral doses of ENT-01 in patients with Parkinson's disease and constipation.

3.1.3 Exploratory Objective

To determine the pharmacodynamic (PD) characteristics of ENT-01 in patients with Parkinson's disease related constipation.

3.2 Objectives for Stage 2

3.2.1 Primary Objectives

- To determine the Safety, Tolerability of repeated oral doses of ENT-01 in patients with Parkinson's disease related constipation.
- To determine the PK and PD characteristics of repeated oral doses of ENT-01 in patients with Parkinson's disease related constipation.

3.2.2 Exploratory Objective

- To determine the effect of study drug on Quality of Life Measures including sleep, temperature, memory, energy, mood, fatigue, hallucinations, memory and executive function in patients with Parkinson's disease related constipation.

4 STUDY ENDPOINTS AND EVALUATIONS

4.1 Pharmacokinetic Parameters

4.1.1 Pharmacokinetic Parameters for Stage 1

The following PK parameters will be determined where possible:

Parameter	Definition
C_{max}	Maximum or peak measured plasma concentration
C_{min}	Minimum or trough measured plasma concentration
T_{max}	Time of maximum or peak measured plasma concentration
$t_{1/2}$	Apparent terminal elimination half-life; calculated as $\ln(2)/ \lambda_z$. 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_{0-24}	Area under the concentration versus time curve from time 0 to the concentration at 24 hours; calculated using linear trapezoid rule
CL/F	Clearance defined as: $Dose/AUC_{0-24}$
λ_z	Apparent terminal rate constant

Additional pharmacokinetic parameters may be determined where appropriate.

4.1.2 Pharmacokinetic Parameters for Stage 2

Pharmacokinetic parameters will not be calculated.

4.2 Safety Evaluations

The safety evaluations for both stages include:

- Treatment emergent adverse events (TEAEs)
- Clinical laboratory assessments
- Vital signs
- Physical examination findings
- Electrocardiogram

4.3 Tolerability Evaluations

Tolerability will be measured by the following DLT endpoints: Dose Limiting Toxicity (DLT) will be the dose which induces repeated vomiting, diarrhea, abdominal pain or symptomatic postural hypotension within 24 hours of dosing. It is defined using the Common Terminology Criteria for Adverse Events (CTCAE) as:

- Recurrent vomiting: 3-5 episodes of vomiting within 24 hours of taking ENT-01 (i.e., 3-5 episodes of Grade 2 vomiting)

- Recurrent diarrhea: 4-6 episodes of diarrhea within 24 hours of taking ENT-01 (will be recoded as a Grade 2 diarrhea)
- Moderate Abdominal pain that limits instrumental activities of daily living within 24 hours of taking ENT-01 (Grade 2 AE)
- postural hypotension including Moderate dizziness, lightheadedness or fainting upon rising from lying to sitting or standing and severe enough to require medical intervention within 24 hours of taking ENT-01 (Grade 2 AE) or either a systolic blood pressure less than 80mmHg or diastolic blood pressure less than 40mm Hg

In addition, the following will also be considered dose limiting toxicity for the purpose of analyses:

- Elevation of LFTs > 3 times the upper limit of normal (ULN)
- A reduction of body weight of 10% or more

4.4 Pharmacodynamic Evaluations

Unless otherwise stated, the following pharmacodynamic (PD) variables are evaluated for both Stage 1 and Stage 2. All PD parameters will be defined for each period. The periods under consideration for summary and analyses are run-in (i.e., baseline), each dosing period or treatment period (as applicable), and washout period (i.e., follow-up period).

For Stage 1 stool diaries, the baseline PD parameters will be calculated using all diaries from run-in period; dosing period PD parameters will be calculated on dosing day immediately following each dose (for CSBM and SBM after dose, need to consider the day before dosing for the rescue medications use); and washout PD parameters will be calculated using diaries excluding the first day diaries (i.e., this one day diaries will be attributed to last dosing period).

For Stage 2 stool diaries, all the diaries for each dosing period will be used for the calculation of PD parameters. The washout period started 24 hours of last dose.

For Stage 1 sleep diaries, PD parameters will be calculated using all diaries from run-in period; up to last dosing period. The first 7-day diaries after last dosing period will be attributed to last dosing period and the second 7-day diaries after last dosing period will be attributed to washout period.

For Stage 2 sleep diaries, PD parameters will be calculated using all diaries from run-in period; all diaries in each dosing period up to last dosing period. The washout period started 24 hours of last dose.

4.4.1 Stool Diary Data

Stage 1 Pharmacodynamics Parameters

- Stage 1 complete spontaneous bowel movement (CSBM) is defined as CSBM (Yes) without rescue meds within 24 hours based on dosing day diary.
- Stage 1 spontaneous bowel movements (SBM) is defined as SBM (Yes) without rescue meds within 24 hours based on dosing day diary
- CSBM days per week will be calculated as follows:
  a. Run-in and washout period: calculation will be based on all diaries in Run-in and Washout period (excluding the first day diary in the period).

$$\text{CSBM Days per Week} = \frac{\text{Sum of days with CSBM without rescue meds within 24 hours}}{\text{Number of Diary Days}} \times 7 \text{ days}$$

  b. Treatment period: calculation will be based on Stage 1 CSBM as:

$$\text{CSBM Days per Week} = \frac{\text{Sum of days with Stage 1 CSBM}}{\text{Number of Dosing Days}} \times 7 \text{ days}$$

- SBM days per week will be calculated as follows:
  a. Run-in and washout period: calculation will be based on all diaries in Run-in and Washout period (excluding the first day diary in the period).

$$\text{SBM Days per Week} = \frac{\text{Sum of days with SBM without rescue meds within 24 hours}}{\text{Number of Diary Days}} \times 7 \text{ days}$$

  b. Treatment period: calculation will be based on Stage 1 SBM as:

$$\text{SBM Days per Week} = \frac{\text{Sum of days with Stage 1 SBM}}{\text{Number of Dosing Days}} \times 7 \text{ days}$$

- Average ease of passage for each period is defined as
  a. Run-in and washout period: calculation will be based on all diaries in Run-in and Washout period (excluding the first day diary in the period).
  b. Treatment period: calculation will be based on diaries on dosing days.

$$\text{Aberage ease of passage} = \frac{Sum\ of\ ease\ scale\ \ w/o\ rescue\ meds\ within\ 24\ hrs}{Number\ of\ BMs\ w/o\ rescue\ meds\ without\ 24\ hrs}$$

- Average stool consistency for each period is defined as

  a. Run-in and washout period: calculation will be based on all diaries in Run-in and Washout period (excluding the first day diary in the period).

  b. Treatment period: calculation will be based on diaries on dosing days.

$$\text{Average stool consistency} = \frac{\text{Sum of stool consistency w/o rescue meds within 24 hrs}}{\text{Number of BMs w/o rescue meds without 24 hrs}}$$

- Days using rescue med per week for each period is defined as

  a. Run-in and washout period: calculation will be based on all diaries in Run-in and Washout periods (excluding the first day diary in the period).

$$\text{Days using rescue meds per Week} = \frac{\text{Sum of days using rescue meds}}{\text{Number of Diary Days}} \times 7 \text{ days}$$

  b. Treatment period: calculation will be based on diaries on dosing days.

$$\text{Days using rescue meds per Week} = \frac{\text{Sum of days using rescue meds}}{\text{Number of Dosing Days}} \times 7 \text{ days}$$

These first two parameters will be treated as categorical variables while other parameters will be treated as continuous variables.

Stage 2 Pharmacodynamics Parameters:

- CSBM days per week for each dosing period is defined as:

$$\text{CSBM Days per Week} = \frac{\text{Sum of days with CSBM w/o rescue meds within 24 hours}}{\text{Number of Diary Days}} \times 7 \text{ days}$$

- CSBM episodes per week for each period is defined as:

$$\text{CSBM epidoses per Week} = \frac{\text{Sum of daily CSBM episodes w/o rescue meds within 24 hours}}{\text{Number of Diary Days}} \times 7 \text{ days}$$

$$\text{where daily CSBM episodes} = \begin{cases} \#\text{of CSBM ocuurences w/o rescue meds within 24 hrs for each diary day} \\ 0 \text{ if none CSBM w/o rescue meds within 24 hrs for each diary day} \end{cases}$$

- SBM days per week for each dosing period is defined as:

$$\text{SBM Days per Week} = \frac{\text{Sum of Days with SBM w/o rescue meds within 24 hrs}}{\text{Number of Diary Days}} \times 7 \text{ days}$$

- SBM episodes per week for each period is defined as:

$$\text{SBM episodes per Week} = \frac{\text{Sum of Daily SBM Episodes w/o rescue meds within 24 hrs}}{\text{Number of Diary Days}} \times 7 \text{ days}$$

$$\textit{where Daily SBM Episodes} = \begin{cases} \#of\ SBM\ occurences\ without\ rescue\ meds\ with\ 24\ hours\ for\ each\ day\ diary \\ 0\ if\ none\ SBM\ without\ rescue\ meds\ for\ each\ day\ diary \end{cases}$$

- Average ease of passage for each period is defined as

$$\text{Average ease of passage} = \frac{\text{Sum of ease scale w/o rescue meds within 24 hrs}}{\text{Number of BMs w/o rescue meds without 24 hrs}}$$

- Average stool consistency for each period is defined as

$$\text{Average stool consistency} = \frac{\text{Sum of stool consistency wi/o rescue meds within 24 hrs}}{\text{Number of BMs w/o rescue meds within 24 hrs}}$$

- Days using rescue meds (i.e., laxative/suppository/enema) per week for each period is defined as

$$\text{Days using rescue meds} = \frac{Sum\ of\ Days\ with\ daily\ rescue\ meds\ use}{Number\ of\ diary\ days} \times 7\ days$$

- Rescue med episodes per week for each period is defined as

$$\text{Rescue med episodes per week} = \frac{\text{Sum of daily rescue meds use}}{\text{Number of diary days}} \times 7 days$$

$$where\ daily\ rescue\ meds\ use = \begin{cases} \#of\ episodes\ \ of\ rescue\ meds\ use\ for\ each\ diary\ day \\ 0\ if\ no\ rescue\ meds\ use\ for\ each\ diary\ day \end{cases}$$

All of the above parameters are treated as continuous variables.

- Success (Yes/No) based on CSBM days will be defined as "Yes" if either one criteria is met:

  a. if increase of one or more in CSBM days per week compared to baseline

  b. 3 or more of CSBM with CSBM per week

Success based on CSBM will be treated as categorical variable and is primary endpoint.

4.4.2 Sleep Diary Data

Sleep diary data are considered as exploratory nature. For both stages, the PD parameters for each period from sleep diary are as below:

- Total sleep time (hours) per night

$$\text{Total sleep time (hrs) per night} = \frac{\text{Sum of daily total sleep time}}{\text{Number of Diary Days}}$$

- Delay (minutes) to sleep onset per night

$$\text{Delay to sleep onset (minutes) per night} = \frac{\text{Sum of daily delay to sleep onset}}{\text{Number of Diary Days}}$$

- Total awake time (hours) per night

$$\text{Total awake time (hours) per night} = \frac{\text{Sum of daily total awake time}}{\text{Number of Diary Days}}$$

- Number of wakes per night

$$\text{Average number of wakes per night} = \frac{\text{Sum of daily number of wakes}}{\text{Number of Diary Days}}$$

- Number of arm or leg thrashing days per week will be calculated as

$$\text{Number of arm or leg thrashing days per Week} = \frac{\text{Sum of days with arm or leg thrashing}}{\text{Number of Diary Days}} \times 7 \text{ days}$$

  Arm or leg thrashing is defined as either arm or leg fidget or leg twitching from sleep behavior question.

Based on number of arm or leg thrashing days per week,

a. Disappearance of arm or leg thrashing from sleep diary – defined as zero days of arm or leg thrashing per week

Except for disappearance of arm or leg thrashing, all the above parameters will be treated as continuous outcomes.

4.4.3 Questionnaire Data

Questionnaire data will be considered as exploratory nature.

4.4.3.1 Non-Motor Symptoms Questionnaire

Non-Motor Symptoms Questionnaire (NMSQ) comprises 30-item questionnaire. The answer for each question is Yes/No (coded as 1/0). The total score will be calculated as a summed score over 30-items [1].

4.4.3.2 Beck Depression Inventory II

Beck depression inventory II (BDI-II) consists of 21 items. Each item is rated on a 4 point scale ranging from 0-3. For Question 16 and 18, the response categories are 0, 1a, 1b, 2a, 2b, 3a, or 3b. For the calculation of total score, the letters a and b will be dropped for these questions.

BDI-II total score will be calculated by summing the ratings for the 21 items based on the manual for Beck Depression Inventory-Second Edition. The maximum total score is 63.

Furthermore, total score will be classified into following categories.

BDI-II Total Scores	BDI-II Depression categories
0-13	Minimal
14-19	Mild
20-28	Moderate
29-63	Severe

4.4.3.3 Mini Mental State Examination

Mini mental state examination (MMSE) consists of 11 types of questions/activity. In each type, the number of questions can range from 1 to 5. Score one point for each correct response for each question or activity within each type.

According to MMSE manual, the total score will be calculated by summing the item scores across all 11 types of questions/activities. The possible maximum total score is 30.

4.4.3.4 The University of Miami Parkinson's Disease Hallucinations Questionnaire

The University of Miami Parkinson's Disease Hallucinations Questionnaire (UM-PDHQ) consists of 6 quantitative questions (Q1 to Q6) regarding severity of hallucinations and 14 qualitative questions regarding quality of hallucination (Q7 to Q20). Question 1 is a gating question to assess the presence or absence of hallucination.

The scoring ranges are not uniform in each item (Q3 and Q4: 0-2; Q2:0-4; Q5: 1-3; and Q6:0-3). According to the information on the questionnaire (total score [min=0, max=14]), the total score from 6 quantitative questions will be calculated by summing the response scores over Q2 to Q6

if the answer of Question 1 is 1 or 2 while total score is equal to zero if the answer for Question 1 is zero (i.e. no hallucinations).

4.4.3.5 Parkinson's disease Fatigue Scale

Parkinson's disease fatigue scale (PFS-16) consists of 16 questions. According to Appendix 15 of protocol, the responses to each question are "Strongly disagree", "Disagree", "Neither agree nor disagree", "Agree", and "Strongly agree". The scoring method used for the study is

PFS-16 Response	Score
Strongly disagree	1
Disagree	2
Neither agree nor disagree	3
Agree	4
Strongly agree	5

The total score will be calculated by summing scores over 16 questions. [2, 3] The possible total scores range from 16 to 80.

4.4.3.6 Patient Assessment of Constipation Symptoms

Patient Assessment of Constipation Symptoms (PAC-SYM) is about constipation in the past two weeks and consists of 12 items. The scores for each item is

PAC-SYM Response	Score
Absent	0
Mild	1
Moderate	2
Severe	3
Very Severe	4

There are total score and 3 subdomain scores: abdominal symptoms (Item 1-4), rectal symptoms (Item 5-7), and Stool symptoms (Item 8-12). Following the information booklet (2nd Edition, May 2015) from Mapi Research Trust, a subscale score is calculated as the average item score of non-missing items within that subscale. Total score is the average score of non-missing items within the instrument.

4.4.3.7 Patient Assessment of Constipation Quality of Life

Patient Assessment of Constipation Quality of Life (PAC-QoL) is to measure the impact constipation has had on subject's daily life over the past 2 weeks.

PAC-QoL: 2 Sets of Responses separated by "/" Depending on questions	Score
Not at all / Never	0
A little bit / Rarely	1
Moderately / Sometimes	2
A lot / Often	3
Extremely / Always	4

PCAC-QoL contains 28 items grouped into 4 subscales: Physical discomfort (Item 1-4, Psychosocial discomfort (Item 5-12), Worries and concerns (Item 13-23), and satisfaction (Item 24-28).

Following the information booklet (4th Edition, May 2015) from Mapi Research Trust, scores of items 18 and 25 to 28 are reversed meaning that the score corresponding to the response:

- "Not at all"/"None of the time" will be 4
- "A little bit"/"A little of the time" will be 3
- "Moderately"/"Some of the time" will be 2
- "Quite a bit"/"Most of the time" will be 1
- "Extremely"/"All of the time" will be 0

Then, subscale score are calculated as the average item scores of non-missing items within that subscale. Total score is calculated as the mean of 28 item score within the instrument. When more than 50% of items are missing for a subscale score, then that subscale score is set to missing. If a subscale is missing, the PAC-QoL total score is set to missing.

4.4.3.8 REM Sleep Behavior Disorder Screening Questionnaire

REM sleep behavior disorder screening questionnaire (RBDQ) comprises 10 items. Each item is answered as yes/no (yes=1 or no =0). According to Scaling and Scoring (Version 1.0: Sept 2016) from Mapi Research Trust, total score will be summed over all items/questions. The maximum score s 13 points. A score of 0 is given to item/question is not answered. The multiple answers for an item will be considered as missing data.

4.4.3.9 Unified Parkinson's disease Rating Scale

Unified Parkinson's disease rating scale (UPDRS) consists of

- Part I: Non-Motor Aspects of Experiences of Daily Living (nM-EDL) - Q1.1 to Q1.6 completed by rater; Q1.7 to Q1.13 completed by subject
- Part II: Motor Aspects of Experiences of Daily Living (M-EDL) - Q2.1 to Q2.13 completed by subject
- Part III: Motor Examination - Q3.1 to Q3.18 completed by examiner
- Part IV: Motor Complications - Q4.1 to Q4.6 by rater

Rating for items is from 0 (normal) to 4 (severe). Score for each part is obtained from the sum of corresponding item scores. Total score is the summed score from the scores of 4 parts,

4.4.3.10 Other Questionnaires

Trail make A& B and Parkinson's disease Sleep Scale (PDSS) will not be used for analyses but will be listed only.

ROME-IV Criteria for Constipation (ROME-IV) is only collected at screening. It will be listed.

5 PLANNED ANALYSES

5.1 Changes from planned analyses in the Protocol

There are no changes from planned Analyses in the Protocol.

5.2 Interim Analyses

After Stage 1 data completed, Stage 1 analyses will be performed.

5.3 Final Analyses and Reporting

All final planned analyses per protocol and this SAP will be performed only after database lock.

6 ANALYSIS POPULATIONS

All data will be presented in listings for the Enrolled population. A subject will be considered enrolled when the subject has been consented, screened, and all eligibility criteria have been confirmed in the electronic case report form (eCRF).

6.1 Pharmacokinetic Population

6.1.1 Pharmacokinetic Population for Stage 1

The Pharmacokinetic (PK) Population will consist of all subjects who received ENT-01 and for whom there are adequate samples collected to provide analyzable PK parameters.

Any subject who violates the protocol in a way which might influence the plasma concentration by time profiles will be excluded from the PK population.

6.1.2 Pharmacokinetic Population for Stage 2

Seven subjects from the ENT-01 group and three subjects from the placebo group will be selected for the Pharmacokinetic (PK) Population.

Any subject who violates the protocol in a way which might influence the plasma concentration by time profiles will be excluded from the PK population.

6.2 Safety Population

For each stage, safety Population will consist of all subjects who receive at least one dose of ENT-01 during that stage.

6.3 Full Analysis Set

For each stage, full Analysis Set (FAS) includes subjects who are treated with ENT-01 in the treatment period and have the stool diary assessments both at baseline and at least one post baseline.

6.4 Randomized population

For Stage 2, randomized population included all subjects who are randomized and receive at least one dose of blinded treatment under the protocol dated 27 July, 2017.

7 STATISTICAL CONSIDERATIONS

All analyses described in this plan are considered a priori analyses in that they have been defined prior to locking the database. All other analyses, if any, designed subsequently to locking the database, will be considered post hoc analyses and will be described as exploratory analyses in the Clinical Study Report.

All summaries and statistical analysis will be performed by SAS® Version 9.3 or later for each stage separately.

7.1 General Statistical Procedures

Frequency distributions for categorical variables will be provided as counts of subjects and percentages. Percentages will be based on the number of subjects with a non-missing parameter unless missing category is presented. Percentages will be reported to one decimal place.

The descriptive statistics for continuous variables will be number of subjects, arithmetic mean, standard deviation (SD), median, quartiles (Q1, Q3), minimum and maximum. Arithmetic mean, median, Q1, and Q3 will be reported to 1 more decimal place than the raw data, while the SD will be reported to two more decimal places than the raw data.

In addition to the above descriptive statistics, the coefficient of variation (CV%) will also be used for the summaries of drug concentration data and pharmacokinetic parameter values. The geometric means will be calculated for AUC and Cmax values.

All data listings that contain an evaluation date will also contain a relative study day.

Missing values will not be imputed.

7.2 Enrollment and Disposition

7.2.1 Subjects Enrollment

Subject enrollment will be summarized for each analysis population separately for each stage. The number of subjects in each analysis population will be presented.

Enrollment information will be provided in a data listing.

7.2.2 Subject Disposition

7.2.3 Subject Disposition for Stage 1

Subject disposition will be summarized for each dose of ENT-01 and overall. Number and percentage of subjects who complete each dose, number and percentage of subjects who discontinued from the study early by primary reasons will be presented. The denominators for calculating the percentages will be based on number of subjects enrolled.

Discontinued subjects will be provided in a data listing.

7.2.4 Subject Disposition for Stage 2

Subject disposition will be summarized for each period and for each analysis population. Number and percentage of subjects who complete the full protocol, number and percentage of subjects who discontinued from the study early by primary reasons will be presented. The denominators for calculating the percentages will be based on number of subjects in the analysis population.

Discontinued subjects will be provided in a data listing.

7.3 Protocol Deviations

Important or significant protocol deviations (PD) will be assessed by sponsor personnel following Protocol Deviation Guideline outlined in Clinical Management Plan.

- A PD is classified as important if there is the potential to impact the completeness, accuracy, and/or reliability of the study data, or affect a subject's rights, safety, or well-being.
- An important PD is classified as significant if it is confirmed to adversely impact the completeness, accuracy, and/or reliability of the study data, or affect a subject's rights, safety, or well-being.

All PDs will be identified and finalized prior to database lock.

All protocol deviations will be listed. Protocol deviations will be presented in summary tables separately for each stage for the Safety population.

7.4 Demographics and Baseline Characteristics

7.4.1 Demographics and Baseline Characteristics

Demographic and baseline characteristics will be summarized overall for each analysis population as applicable.

The demographic data are:

- Age
- Sex
- Race
- If female, childbearing potential (Yes/No)
- Ethnicity
- Height (cm)

- Baseline Weight (kg)
- BMI (Body mass index, kg/m^2, calculated as Weight (kg) / Height (m)^2)

Conversions for height and weight are as follows:

Height (cm) = Height (inches) x 2.54

Weight (kg) = Weight (lb) x 0.4536

The demographic data will be listed.

7.4.2 Baseline Disease Characteristics

Age at diagnosis of Parkinson's disease, years with Parkinson's disease, years of constipations, Hoehn and Yahr stage, and constipation severity – CSBM will be summarized as appropriate for each analysis population.

Baseline disease characteristics will be provided in a listing.

7.4.3 Medical History

In Stage 2, general medical history will be collected. The medical history will be coded using the current version of the Medical Dictionary for Regulatory Activities (MedDRA). The frequency count and percentage of patients experiencing any medical conditions will be tabulated by MedDRA system organ class (SOC) and preferred term (PT) for the Safety and Randomized population. The denominators for calculating the percentages will be based on number of patients in the analysis population.

A data listing of medical history will be provided.

7.5 Prior and Concomitant Medications

All medications as documented by the investigator will be coded using the World Health Organization (WHO) Drug Dictionary. The count and percentage of subjects who take prior/concomitant medications will be provided using therapeutic class and WHO Drug preferred name. The denominators for calculating percentages will be based on the number of subjects overall in the Safety population. For the summary tables, if a subjects took a prior or concomitant medication more than once, the subject would be counted only once for each therapeutic class and preferred name.

Prior medications are defined as medications taken prior to the first dose of study drug (ENT-01). Concomitant medications are defined as medications taken on or after the first dose study drug. Prior and concomitant medications will be summarized overall for the safety population.

For Randomized population, concomitant medications will be summarized by treatment.

All prior and concomitant medications, including ancillary medication will be listed.

7.6 Analysis of Pharmacokinetic Data

7.6.1 Summary of Pharmacokinetic Concentration Data for Stage 1

All plasma concentration data will be summarized based on the PK population by timepoint (pre-dose, 1, 2, 4, 8 and 24 hours post-dose) and dose level in terms of n, mean, SD, CV%, median, minimum, maximum, geometric mean, and geometric CV%.

Individual plasma concentration will be listed by timepoint and dosage. The concentration will be listed to the same degree of accuracy as supplied by the analytical laboratory and PK group. As well, the units of concentration will be presented as they are received from the bioanalytical laboratory and PK group.

Individual concentration and mean plasma concentration will be displayed graphically.

Summary of Pharmacokinetic Concentration Data for Stage 2

Individual plasma concentration will be listed by timepoint for the PK population. The concentration will be listed to the same degree of accuracy as supplied by the analytical laboratory and PK group. As well, the units of concentration will be presented as they are received from the bioanalytical laboratory and PK group.

Individual concentration will be displayed graphically using actual sampling times.

7.6.2 Analyses of Pharmacokinetic Parameters

7.6.3 Analyses of Pharmacokinetic Parameters for Stage 1

Summary of PK parameters for each dose level will be presented in terms of n, mean, SD, CV%, median, minimum, maximum, geometric mean, and geometric CV%. Geometric mean and geometric CV% will only be calculated for AUC and Cmax. Individual derived PK parameters will also be listed by dose level.

7.6.4 Analyses of Pharmacokinetic Parameters for Stage 2

No PK parameters will be derived.

7.7 Analysis of Pharmacodynamics Data

Generally speaking, for Stage 1 and Stage 2, the summaries will be provided for active treatment ENT-01 or by dose level if deemed as necessary. The comparison is made to baseline for each PD variable up to the end of fixed dosing period plus washout period.

For Randomized population of Stage 2, the descriptive summaries will be provided for active treatment and placebo. Due to small sample size resulting from amended protocol, there will be no formal statistical comparisons between active treatment and placebo for PD variables.

7.7.1 Analysis of Continuous Pharmacodynamics Data

The following continuous PD parameters and their change from baseline will be summarized by period (run-in [baseline), dosing periods (diary) or treatment period (questionnaire data), and washout) for each appropriate population, using descriptive statistics as specified in Section 7.1 (General Statistical Procedures).

Stool Diary PD parameters:

- CSBM days per week
- CSBM episodes per week (Stage 2 only)
- SBM days per week
- SBM episodes per week (Stage 2 only)
- Average ease of passage
- Average stool consistency
- Days using rescue meds per week
- Number of rescue meds use per week

Sleep Diary PD parameters:

- Total sleep time (hours) per day
- Delay (minutes) in sleep onset per night
- Total awake time (hours) per night
- Number of wakes per night
- Number of arm or leg thrashing days per week

Questionnaire PD parameters:

- Total score from NMSQ
- Total score from BDI-II
- Total score from MMSE
- Total score from UM-PDHQ
- Total scores from PFS-16
- Total score and 3 subdomain scores from PAC-SYM
- Total Score and 4 subdomain scores from PAC-QoL
- Total Score from RBDQ
- Total score and Part I to III scores from UPDRS

7.7.2 Analysis of Categorical Pharmacodynamics Data

The following categorical PD parameters will be summarized by period (run-in [baseline], dosing periods [diary] or treatment period [questionnaire data], and washout) for each

appropriate population, using frequency distribution as specified in Section 7.1 (General Statistical Procedures).

Stool Diary PD parameters:

- Stage 1 CSBM based on dosing day diary
- Stage 1 SBM based on dosing day diary
- Success based on CSBM days per week (Stage 2 only) - primary efficacy outcome variable
    - The proportion of subjects for whom the drug was a success at the end of fixed period will be estimated with a binomial point estimate and corresponding 95% confidence interval. The observed proportion of successes will be compared to 0.10 using an exact binomial test. Success will be defined as an increase in CSBM/week of 1 or more OR a CSBM/week of 3 or more.

Sleep Diary PD parameters:

- Disappearance of arm or leg thrashing (Yes/no)

Questionnaire PD parameters:

- BDI-II Depression categories

7.8 Analysis of Safety Data

7.8.1 Adverse Events

Adverse events will be coded into SOC and PT using the current version of MedDRA. Severity of AEs will be assessed by investigators according to CTCAE (v4.03). In database, Grade 1 is labeled as Mild, Grade 2 as Moderate, and Grade 3 as Severe.

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 number and percentage of subjects who experienced an AE during escalation and fixed dosing periods will be summarized by dose level (Stage 1: 75 mg up 200 mg; Stage 2: 75 mg up to 250 mg, fixed dose, and washout) and overall for each stage and for the Safety population. The denominator for calculating the percentages will be based on the number of subjects ever exposed to each dose and overall in the Safety Population.

Additionally, the number and percentage of subjects who experienced an AE during randomization period will be summarized by treatment (Placebo and ENT-01). The denominators for calculating the percentages will be based on the number of subjects ever exposed to each treatment in the Randomized Population.

A treatment emergent adverse event (TEAE) is defined as adverse events with a date of onset (or worsening) on or after the start date of a treatment up to the start date of the next treatment. 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 discontinuation by SOC and PT
- TEAEs of special interest including nausea, diarrhea, and dizziness by SOC and PT.

If a preferred term or system organ class was reported more than once for a subject, the subject would only be counted once in the incidence for that preferred term or system organ class.

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.

Adverse events including baseline AEs, serious adverse events, and adverse events leading to discontinuation from the study will be provided in separate data listings.

7.8.2 Clinical Laboratory Tests

Stage 1 and up to end of fixed dosing period in Stage 2

In this study, clinical laboratory tests were done at local laboratories. Therefore, the reference ranges may not be the same. All laboratory test results will be summarized by period (run-in period, dose period, and washout period) using frequency distribution for low/normal/high or normal/abnormal. The denominators for calculating the percentages will be based on the number of subjects with non-missing values in the Safety Population.

Shift from baseline in laboratory parameters will be summarized by period for the Safety Population by period based on Low/Normal/High classification. The denominators for calculating the percentages will be based on the number of subjects with non-missing values at both baseline and each post-baseline analysis visit in the Safety Population.

Randomization period in Stage 2

Additionally, the above summaries will be provided for viral signs during the randomization period by treatment for randomized population.

All clinical laboratory assessments will be listed.

7.8.3 Vital Signs

Stage 1 and up to end of fixed dosing period in Stage 2

Vital signs (Stage 1 and up to end of fixed dosing period in Stage 2) for body temperature, respiration rate, heart rate and weight will be summarized by period, together with the change from baseline. Baseline for body temperature, respiration rate, heart rate and weight will be the last assessment during the run-in period.

Systolic and diastolic blood pressure will be summarized by time point (pre-dose and 2 hours post-dose) and dose period separately for each position (lying down and sitting). Baseline for predose in each dose period and washout will be the last assessment during the run-in period. Baseline for 2 hour post dose in each dose period will be the assessment at predose at that dose period.

All summaries will be provided for the safety population.

Randomization period in Stage 2

Additionally, the above summaries will be provided for viral signs during the randomization period by treatment for randomized population.

7.8.4 Electrocardiogram (ECG)

Stage 1 and up to end of fixed dosing period in Stage 2

ECG findings will be classified as normal vs abnormal. The number and percentage of each category will be summarized using frequency table for run-in, dose period, and wash-out. The denominators for calculating the percentages will be based on the number of subjects with non-missing values in each period for the Safety Population. Baseline will be defined as the most recent measurement taken in the run-in period.

Randomization period in Stage 2

Additionally, the above summaries will be provided for ECG during the randomization period by treatment for randomized population.

Detailed ECG results will be provided in a data listing.

7.8.5 Physical Examination

Stage 1 and up to end of fixed dosing period in Stage 2

Summary of physical examinations will present frequency distribution by body system and each period. The denominators for calculating the percentages will be based on the number of subjects evaluated for a particular body system of each dose level in the Safety Population.

Randomization period in Stage 2

Additionally, the above summaries will be provided by treatment for randomized population.

Details on physical examinations will be provided in a data listing.

7.8.6 Analysis of Tolerability Data

Stage 1 and up to end of fixed dosing period in Stage 2

Tolerability will be measured by DLT endpoints defined in Section 4.3.

The frequency of each tolerability endpoints including recurrent vomiting, recurrent diarrhea, abdominal pain and postural hypertension, and etc. will be summarized by dose (Stage 1) or for treatment period (Stage 2). Percentages will be based on the number of subjects in each dose level (Stage 1) or the number of subjects (Stage 2) in the safety population.

Randomization period in Stage 2

Additionally, the above summaries will be provided during the randomization period by treatment for randomized population.

7.8.7 Extent of Exposure

For Stage 1, dose administration details by subject will be listed.

For Stage 2, number of subjects and percentage on each dose level will be presented for period 3.2. The denominators for percentages will be based on the number of subjects in the Safety Population. For each dose period, total dose (mg), extent of exposure (days), and applicable compliance (%) captured on the eCRF page will be summarized using descriptive statistics.

7.8.8 I-BUTTON

I-Button data will be analyzed by a separate group. The data will not be included in this SAP.

7.9 Additional Data Presentation as Listing

Additional data listings will be provided for the following information as appropriate:

- Subject eligibility
- Medical history
- Constipation history
- Stool diary
- Sleep diary
- Individual questions from questionnaires:
    - NMSQ
    - BDI-II
    - MMSE
    - UM-PDHQ
    - PFS-16
    - PAC-SYM
    - PAC-QoL
    - RBDQ
    - UPDRS
    - ROME-IV
    - Trail make A
    - Trail make B
    - PDSS

8 DEFINITIONS AND CONVENTIONS FOR DATA HANDLING

8.1 Definition of Baseline

For all evaluations unless otherwise noted, baseline is defined as the most recent measurement prior to the first administration of study drug in Stage 1. Baseline can be the same date as first dose, given the measurement is expected prior to first dose when only date information is available.

8.2 Analysis Visit Window

For safety parameters excluding clinical laboratory data, measurements collected from unscheduled visits will not be included in the by-visit summary tables but will be included in the listings. Early termination visits for safety measurements will not be mapped to any scheduled post-baseline visit, but will be used as the last assessment during treatment period.

8.3 Pharmacodynamic Data Handling

All PD parameters from questionnaires will follow instructions or manuals coming in with them. If any special handling rules were needed, they would be documented in ADaM data specifications.

For PD parameters from diary data, generally, there will be no imputation for missing diaries. However, if there were some partial dates or times not in 24-hour clock present in diary data, they might be imputed in order to derive PD parameters, using common sense. These details of handling rules would be documented in ADaM data specifications.

8.4 Safety Data Handling

For all safety data, only observed data will be used for analyses, and missing data will not be imputed.

8.5 Handling of Repeated Clinical Laboratory Tests

For laboratories results at unscheduled visits, it will be treated as repeated laboratory results for the closest previous visit. The last repeat of laboratory results will be used in the summary tables for that visit. All the laboratory test results (original test results and repeated results) will be included in the data listings.

8.6 Pharmacokinetic Data Handling

8.6.1 Pharmacokinetic Plasma Drug Concentration Data

Pharmacokinetic concentration values below the quantification limit (BLQ) will be treated as zero for the calculation of summary statistics.

Individual values that are BLQ will be presented as "BLQ" in the concentration data listing.

No imputation will be performed for missing concentration.

8.7 Handling of Partial Dates for AEs

When determining the treatment emergent AE, partial dates will be handled as follows.

- If the day of the month is missing, the onset day will be set to the first day of the month unless it is the same month and year as first dose date. In this case, the onset date will be assumed to be the first date of treatment.
- If the onset day and month are both missing, the day and month will be assumed to be January 1, unless the event occurred in the same year as the study treatment. In this case, the event onset will be coded to the day of treatment to conservatively report the event as treatment-emergent.
- A missing onset date will be coded as the day of treatment. If the resulting onset date is after a reported date of resolution, the onset date will be set equal to the date of resolution.
- Imputation of partial dates is used only to determine whether an event is treatment-emergent; data listings will present the partial date as recorded in the eCRF.

8.8 Handling of Partial Dates for Medications

A medication with a completely missing start date will be considered a prior medication. A medication with a completely missing stop date will be considered to be ongoing.

When determining prior or concomitant medications, partial start dates of prior and concomitant medications will be assumed to be the earliest possible date consistent with the partial date. Partial stop dates of prior and concomitant medications will be assumed to be the latest possible date consistent with the partial date.

9 Changes to the Planned Analyses in the Protocol(s)

Because protocol amendment dated 02 February 2018 removed randomization portion during the trial, there will not be enough samples size to do the following analysis specified in the original protocol dated dated 27 July 2017. Therefore, the following analysis will not be performed for CSR.

- A secondary analysis will compare the proportions of subjects who are deemed a success at the end of the randomized fixed-dose period between those randomized to the ENT-01 arm and those randomized to the placebo arm. Subjects to be included in this analysis will only be those who have been randomized. If the number of subjects for this analysis is between 20 and 30, a Fisher's exact test will be used to compare the proportions of subjects who are deemed a success at the end of randomization period between the two randomized arms.

The protocol also proposed the following analysis as below:

- Another secondary analysis will compare the proportions of subjects who are a success (compared to baseline) between the end of the first week of the washout period (period 5) and the end of the fixed-dose period (period 4). This analysis will consist only of those subjects with assessments at baseline (end of run-in period, Period 3.1), the end of Period 4, and the end of Period 5. If the number of subjects for this analysis is between 17 and 20 subjects, this analysis will be done using an exact sign test of equality of paired proportions.

In this analysis, using the end of the first week of washout is not consistent with the time frame for other PD parameters (note: other PD parameters' are all evaluated using two weeks of washout data, i.e., the end of 2 weeks). Therefore, it was determined we would not do this analysis for CSR.

10 REFERENCES

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