Protocol Title:

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

Sponsor
Enterin, Inc.
2005 Market Street, Suite 3125
Philadelphia, PA 19103

Investigational Product
ENT-01

Study Phase
2b

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ENT-01-030

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STUDY NUMBER ENT-01-030
AMENDMENT NUMBER 7

SUMMARY OF CHANGES

Section Number	Summary of Changes	Rationale for Change
Section 4.0, Study Synopsis	"80 subjects" changed to "up to 80 subjects"	To correspond with "60 evaluable subjects"
Section 4.0 Study Synopsis	"20 centers" changed to "25 centers"	Slow recruitment during Covid
Section 5.3 Study Synopsis	Requirement to take OTC laxatives for 6 weeks prior to inclusion removed; duration of constipation required changed to approximately 6 months	Unnecessary; subjects with more than 3 CSBMs per week do not proceed to randomization anyway.
Study Synopsis	Discontinuation of clonazepam removed	Sleep no longer being assessed
Section 5.4, 5.7 Study Synopsis	Discontinuation of pimavanserin removed	Subjects reluctant to participate and PIs feel it is an unnecessary exclusion
Section 6.2 Packaging and Labeling	Picture of actual label inserted to replace the previous picture	Provides a picture of the actual label and removable panel.
Section 6.3 Storage, Dispensing and Reconciliation of Study Drug and Identity of Investigational Products	Inserted "Controlled room temperature" as the storage temperature requirement. A definition of Controlled room temperature is also provided.	Allows for the minor temperature excursions outside the 20 - 25°C temperature range often experienced in the storage of IP at hospitals, pharmacies and clinical sites.
Section 8.2 – NCI-CTCAE Table	Definitions of Dose Limiting Adverse Events - Vomiting	Language was confusing to coordinators. More precise wording
Section 8.10	Reporting of Adverse Events (AEs) or Serious Adverse Events (SAEs) – AE's and SAE's begins after obtaining of informed consent	To clarify the inconsistency within the Protocol
Section 9.2.5 Study Synopsis	Sample size assumptions reworded to include standard deviation and 10% drop out rate assumption; sample size unchanged	More precise wording
Section 9.2.6 Study Synopsis	Data cut will be performed at 40 subjects changed to data cut may be performed at 40 subjects	To correspond to SAP
Study Synopsis Section 9.2.6	Mentioned that we performed an interim analysis earlier	Also mentioned in earlier amendment

Section 4.0, 4.1,4.2,4.3,4.5,4.6	Visit window broadened	Was too narrow; was leading to many, unnecessary minor protocol deviations
Section 4.1	Specifying that SAPS-PD and hallucination interview only need to be remeasured if hallucinations were present at baseline	Eliminating unnecessary questionnaires
Section 4.2	Subjects allowed to continue stool diary longer to enable 11 days of data collection prior to randomization	Several subjects needed a few extra days to collect the required data, especially those with cognitive impairment
Section 4.2, 4.3,4.4, 4.5, 4.6,	Combined SAPS-PD and hallucination questionnaire	To keep hallucination related questions together
Section 4.1, 4.2, 4.3, 4.4, 4.5	L-Dopa sub-study related language clarified	Was confusing to coordinators
Section 4.1, Table 1 footnote, Appendix 2	Language relating to orthostatic blood pressure measurements clarified.	
Section 3.3, 4.0, 4.1	UPDRS certification requirement removed	Covid is making it difficult to recertify coordinators every 6 months

CONFIDENTIALITY STATEMENT

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

PROTOCOL ENT-01-030 AMENDMENT

Investigator Agreement

I have read this protocol amendment "A Phase 2b Multicenter, Randomized, Double-blind,Placebo-controlled, Multiple Dose Study to Evaluate Safety, Tolerability, and Efficacy of Orally Administered ENT-01 for the Treatment of Parkinson's Disease (PD)-related Constipation (KARMET)" and any auxiliary materials related to Study ENT-01-030 and agree to the following:

1. To conduct the study as described in the protocol, protocol amendment, and any auxiliarymaterials
2. To protect the rights, safety, and welfare of the participants in the study
3. To provide oversight to all personnel to whom study activities have been delegated
4. To maintain control all investigational product (IP) provided by the Sponsor and tomaintain records of the disposition of those products
5. To conduct the study in accordance with the protocol amendment and all applicable localand national regulations, including, but not limited to, the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice (GCP), and the standards set forth by the ethical principles that have their origin in the Declaration of Helsinki, GCPsas indicated in Food and Drug Administration (FDA)
   ICH E6 (R2) and ICH E3.
6. To obtain approval for the protocol, protocol amendment, and all written materialsprovided to participants prior to initiating the study at my site
7. To obtain informed consent from all participants enrolled at my study site prior to initiatingany study-specific procedures or administrating IP to participants
8. To maintain accurate records of each subject's participation

_____

Investigator's Signature Date

STATEMENT OF COMPLIANCE

The study will be conducted in compliance with this clinical study protocol. Good Clinical Practices(GCP) pursuant to ICH E6 (R2), and all applicable local and national regulatory requirements.
Enrollment at any clinical study site may not begin prior to that site receiving approval from the ethicscommittee/IRB of record for the protocol and all materials provided to potential participants.

Any amendments to the protocol or changes to the consent document will be approved before implementation of that amendment. Reconsent of previously enrolled participants may be necessarydepending on the nature of the amendment.

The Principal Investigator (PI) will ensure that changes to the study plan as defined by this Protocol willnot be made without prior agreement from Enterin, Inc and documented approval from the ethics committee/IRB or record, unless such a change is necessary to eliminate immediate hazard to the study participants.

All personnel involved in the conduct of this study have completed Human Subjects Protection and GCPTraining as outlined by their governing situation.

STUDY CONTACT INFORMATION

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SPONSOR SIGNATURE PAGE

PROTOCOL APPROVAL PAGES(S)

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LIST OF ABBREVIATIONS

ADLs	Activities of Daily Living
AE	Adverse Event
BDI-II	Beck Depression Inventory II
CNS	Central nervous system
CRO	Contract Research Organization
CSBM	Complete spontaneous bowel movement, defined as a bowel movement (BM) that did not occur within 24 hours following use of rescue medication, where the subject felt that he/she had no more stool to pass and was completely emptied out.
C-SSRS	Columbia-Suicide Severity Rating Scale
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Tolerability
DSMB	Data Safety and Monitoring Board
eCRF	Electronic Case Report Form
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ENS	Enteric nervous system
EoS	End of study
EoT	End of Treatment
FDA	Food and Drug Administration
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
GI	Gastrointestinal
ICF	Informed Consent Form
IRB	Institutional Review Board
IRT	Interactive Response Technology. IRT includes Interactive Voice Response system and Interactive Web Response system
IWRS	Interactive Web Response System
L-Dopa	Levodopa
LFT	Liver function test

MDS-UPDRS	Movement Disorder Society's Unified Parkinson's Disease Rating Scale
mITT	Modified Intent-to-treat
MMSE	Mini Mental State Examination
MTD	Maximum Tolerable Dose
PAC-QOL	Patient Assessment of Constipation - Quality of Life
PAC-SYM	Patient Assessment of Constipation Symptoms
PD	Parkinson's Disease
PDSS	Parkinson's Disease Sleep Scale
PFS	Parkinson's Disease Fatigue Scale
RBDQ	REM Sleep Behavior Disorder Questionnaire
REM	Rapid eye movement
SAE	Serious Adverse Event
SAPS-PD	Scale for Assessment of Positive Symptoms – Parkinson's Disease
SBM	Spontaneous bowel movement, defined as any bowel movement (BM)that did not occur within 24 hours following use of rescue medication
TEAE	Treatment Emergent Adverse Event
ULN	Upper Limit of Normal
UM-PDHQ	University of Miami Parkinson's Disease Hallucination Questionnaire
UPDRS	Unified Parkinson's Disease Rating Scale
WHO	World Health Organization

1 STUDY SYNOPSIS

Study Title	A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Multiple Dose Study to Evaluate Safety, Tolerability and Efficacy of Orally Administered ENT-01 for the Treatment of Parkinson's Disease-Related Constipation (KARMET)
Protocol Number	ENT-01-030
Study Phase	2b
Methodology	This first part of this 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. Approximately 72 subjects were randomized 3:1 to treatment or placebo (double-blind), with approximately 54 subjects allocated to the treatment groups and approximately 18 to placebo. In this amendment, up to an additional 80 subjects (approximately) will be randomized 1:1 to treatment or placebo (double-blind) with approximately 40 subjects allocated to each group. Randomization will be stratified on baseline weekly CSBM rate. Subjects with a baseline weekly CSBM rate of 1-3.0 will start with 3 tablets daily (3 placebo tablets or equivalent to 75 mg of ENT-01 if randomized to active treatment), while subjects with a baseline weekly CSBM rate of 0 - 0.9 will start with 6 tablets daily (6 placebo tablets, or equivalent to150 mg of ENT- 01 if randomized to active treatment). Dose will then be increased by 25 mg (or 1 placebo tablet) every three days, until an effective dose is reached, up to a maximum dose of 10 tablets (i.e., 250 mg of ENT-01 if randomized to active treatment) or maximum tolerated dose (MTD) is reached, whichever comes first. 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. The study will be conducted on an out-patient basis. Each subject will have 6 visits to the clinic: Visit 1/Screening Visit; Visit 2/RandomizationVisit; Visit 3/Follow up Visit (during the dosing period); Visit 4/Single- Blind Placebo Withdrawal Period Visit; Visit 5/Washout Period; and Visit 6/ End of Study Visit.
Study Duration	Each subject will participate in the study for approximately 10 weeks. The duration of the amended study, from first subject enrolled to last subject last visit, is anticipated to be 6 to 9 months.
Study Centers	This is a multicenter trial at up to 25 U.S. sites.

Objectives	**Primary Objectives:** 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 subjects 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. **Secondary Objectives:** • To determine if the correct starting dose (75 mg or 150 mg) 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). **Exploratory Objectives:** • To determine any change 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, 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
Number of Subjects	The study will have approximately 152 randomized subjects, with up to approximately 80 subjects being randomized in this extension and captured in this amendment.
Study Description	Multicenter, randomized, double-blind, placebo-controlled, study of daily oral doses of ENT-01 for up to 25 days
Study Population	Parkinson's Disease with constipation
Study Drug Administration	Oral
Inclusion/Exclusion Criteria	The study population is defined as subjects who meet the following criteria: Inclusion Criteria:

	1. Subjects aged 30-90 years, both genders. 2. Subjects must provide written informed consent and be willing and able to comply with study procedures. 3. Subjects must be diagnosed with Parkinson's Disease defined as the presence of at least three of the following cardinal features, in the absence of alternative explanations or atypical features: rest tremor, rigidity, bradykinesia and/or akinesia, postural and gait abnormalities. 4. There are insufficient criteria for Irritable Bowel Syndrome (IBS). 5. Constipation which has been present for approximately 6 months. 6. Body mass index (BMI) of 18-40 kg/m^2. 7. Subjects must fulfill Rome IV criteria for functional constipation which includes 2 or more of the following: • Straining during at least 25% of defecations • Lumpy or hard stools in at least 25% of defecations • Sensation of incomplete evacuation for at least 25% of defecations • Sensation of anorectal obstruction/blockage for at least 25% of defecations • Manual maneuvers to facilitate at least 25% of defecations (e.g., digital evacuation, support of the pelvic floor) 8. Self-report of fewer than 3 complete spontaneous bowel movements (CSBMs) per week 9. Loose stools are rarely present without the use of laxatives. 10. Subjects must be able to read, understand, and accurately record data into the diary to guarantee full participation in the study. 11. Female subjects must have negative serum or urine pregnancy tests and must not be lactating. For females able to bear children, hormonal (i.e., oral, implantable, or injectable), abstinence, same sex relationships, single-barrier method, or a double-barrier method of birth control must be used throughout the study. A vasectomized partner will be allowed as one in conjunction with another single-barrier method. 12. Female subjects unable to bear children must have this documented in the CRF (i.e., tubal ligation, hysterectomy, or postmenopausal [defined as a minimum of one year since the last menstrual period]). Post-menopausal status will be confirmed by follicle stimulating hormone (FSH) in women less than 60 years of age. Exclusion Criteria: 1. Unable or unwilling to provide informed consent or to comply with study

	procedures. 2. Diagnosis of secondary constipation beyond that of Parkinson's Disease. 3. Review of Screening Period diaries indicates either of the following: • Fewer than 11 days of diary completion • More than 3 complete spontaneous bowel movements per week based upon the average CSBM rate reported during the Screening Period. 4. A compromised gastrointestinal system which includes: • Structural, metabolic, or functional GI diseases or disorders • Acute GI illness within 2 weeks of the screening visit • History of major GI surgery within 30 days of the screening visit (a history of cholecystectomy, polypectomy, hernia repair or appendicectomy are not exclusionary as long as they were performed more than 30 days before the screening visit). 5. Unable or unwilling to withdraw from laxatives, opiates,or any medications which may cause constipation 2 weeks prior to the dose adjustment period and throughout the rest of the study. 6. Unable or unwilling to withdraw from proton pump inhibitors and antacids at the end of the screening period. 7. Any clinically significant abnormalities on screening laboratories or physical examination requiring further evaluation or treatment. 8. Neurological disorder other than Parkinson's Disease that in the opinion of the investigator might interfere with the conduct of the study. 9. On treatment with intra-jejunal dopamine or carbidopa/levodopa (i.e. Duopa). 10. Subjects starting a new Parkinson's Disease medication or modifying an existing medication within 2 weeks prior to enrollment. 11. Unable to maintain a stable diet regimen. 12. Subjects with a cognitive impairment that preclude them from understanding the informed consent. 13. Subjects placed under legal guardianship. 14. Females who are pregnant or breastfeeding. 15. History of excessive alcohol use or substance abuse. 16. Participation in an investigational drug trial within the month prior to dosing in the present study. 17. Any other reason, which, in the opinion of the investigator, would confound proper interpretation of the study.
Study Product, Dose, Route, Regimen	ENT-01 and placebo will be administered in tablet form, once daily, on an empty stomach, first thing in the morning, with 8 oz of water. No food

	intake will be allowed for 60 minutes after taking medication or placebo. For subjects randomized to active treatment, their tablets will be 25 mg of ENT-01 each. Subjects randomized to active treatment with a baseline weekly CSBM rate of 0-0.9 will begin at 150 mg of ENT-01 and subjects randomized to active treatment with a baseline weekly CSBM rate of 1.0-3.0 will begin at 75 mg of ENT-01. Subjects randomized to placebo with a baseline weekly CSBM rate of 0-0.9 will begin at 6 placebo tablets and subjects randomized to placebo with a baseline weekly CSBM rate of 1.0-3.0 will begin at 3 placebo tablets.
Study Endpoints	**Primary Endpoints:** **Primary Safety Endpoints:** Adverse events as evaluated with subject self-reporting, vital signs, clinical chemistry and hematology analyses on blood samples, urinalysis, and electrocardiograms (ECG). **Primary Tolerability Endpoints:** • Recurrent vomiting. • Recurrent diarrhea. • Abdominal pain. • Dizziness. **Primary Efficacy Endpoint:** Change from baseline in the weekly CSBM rate during the Fixed Dose period. **Secondary Efficacy Endpoints:** • Frequency of dose adjustments in the Dose Adjustment period. • Prokinetic bowel response during the Fixed Dose period. • Change from baseline in the weekly SBM rate during the Fixed Dose period. • SBM bowel response to ENT-01 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. • 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. • Stool-related subjective assessments using the PAC-SYM and PAC-QOL. **Exploratory Efficacy Endpoints:**

	• 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. • 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 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 a SAPS-PD score >0 at the Screening and Randomization visits). • Improvement in symptoms of Parkinson's Disease as assessed by the Movement Disorder Society UPDRS (MDS-UPDRS). • Improvement in mood as assessed by the Beck Depression Index (BDI-II). • Improvement in cognition as assessed by the MMSE. • Change in plasma L-Dopa levels 20, 40, and 60 minutes after L- Dopa and ENT-01 dose. • Change in stool microbiome during treatment.
Statistical Analysis	**Sample Size Consideration** Sample size for the first portion of 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. In this protocol amendment, enrolling up to an additional 80 subjects in a 1:1 randomization will yield a conditional power of approximately 93% and an additional 60 subjects will yield a conditional power of 88%. This is based on the additional subjects having a least squares mean change from baseline for the Fixed Dose Period of 3.55 and 1.28 for ENT-01 and placebo, respectively, and a common standard deviation of 3.88. The power calculations incorporated a 10% drop-out rate. These parameter estimates

	and conditional power calculations are based on the results of the initial cohort of subjects in this Phase 2b study.
	Analysis Populations • Safety Population: The Safety Population will consist of all subjects who receive at least one dose of study medication during the study. • 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. • 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. • 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. **Analysis Methods** Descriptive summary statistics will be presented for the safety and tolerability endpoints by treatment group and overall. Continuous variables will be summarized using the number of observations, mean, standard deviation, median, minimum, and maximum. Categorical variables will be summarized using frequency counts and percentages. 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 Efficacy Evaluable 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 (active or placebo) as a factor, and a subject's baseline weekly CSBM rate as a covariate. The comparison of the active versus the placebo treatment group will be at the two-sided 0.05 significance level. **Interim Analysis** An unblinded interim analysis was performed after the initial cohort of the study. A blinded second interim analysis may be performed after the 40th subject (in the 1:1 randomized cohort) completes the Fixed Dose period (i.e. Visit 4).
Initial Date	December 2018
Completion Date	November 2021

Table 1. Study 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 ofStudy	Early Termination
	Day -21	Day 1 (+/-8 days)	Day 11 (+/- 3 day)	Day 26 (- 2 days)	Day 39 (+/- 8 days)	Day 69 (+/- 8 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[e]	X[e]	X[e]	X[e]	X[e]	X[e]
IP/Placebo Dispense		X	X	X			
IP/Placebo Collection and Accountability			X	X	X	X	X

Commented [A1]: Confirm whether +/- 8 days or only +8 days

	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 -21	Day 1 (+/-8 days)	Day 11 (+/- 3 day)	Day 26 (- 2 days)	Day 39 (+/- 8 days)	Day 69 (+/- 8 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			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 be taken in the supine position and will include BP and HR, sitting or standing BP and HR, RR, and body temperature. . Sitting or standing measurements will be taken within approximately 5 minutes of sitting or standing.

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 (IWRS) 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 and no solid food will be taken for another 60 minutes. Subjects will remain in a sitting upright or standing position for at least 60 minutes after taking medication.
   - 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, subjects will reach the "on" state earlier or later than at 60 minutes. In those cases, MDS-UPDRS should be performed when the Subject 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 subjects discontinue from the study prior to the EoT visit, a stool sample should be collected at the Early Termination visit (as applicable).

AE=adverse event; BDI-II= Beck Depression Inventory II; BM=bowel movement; BP=blood pressure; C-SSRS= Columbia-Suicide Severity Rating Scale; ECG=electrocardiogram; EoT=end of treatment; FSH=follicle-stimulating hormone; HR=heart rate; L-Dopa=levodopa; MDS-UPDRS=Movement Disorder Society's Unified Parkinson's Disease Rating Scale; MMSE=Mini-mental State Examination; PAC-QoL=Patient Assessment of Constipation - Quality of Life;

PAC-SYM=Patient Assessment of Constipation Symptoms; PK=pharmacokinetic(s); RR=respiratory rate; SAPS-PD=Scale for Assessment of Positive Symptoms – Parkinson's Disease; UPT=urine pregnancy test.

2 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. In North America, 63M people meet the Rome IV criteria for constipation and in the US alone, constipation is responsible for over 2M physician visits annually. Laxatives are prescribed to 2- 3M patients every year and furthermore, in most patients, the condition is chronic requiring life- long treatment (Bouras and Tangalos, 2009).

Constipation is much more common among patients with Parkinson's Disease (PD) than in the general population. There are 1M 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). This represents an economic burden to the individual with Parkinson's Disease and to the healthcare system. According to the most recent Federal Supply Schedule (FSS; April 2016), the average 30- day reimbursed price for a basket of orally administered drugs for constipation is approximately $260 or $3120 per year. This represents about $1.8B of prescription laxatives just for patients with Parkinson's Disease. Constipation not only constitutes a major economic burden, but it also significantly affects the quality of life of the individual with Parkinson's Disease, contributing to social isolation and depression. Furthermore, the severity of the symptoms correlates negatively with patient reported quality of life.

An effective prokinetic medication for individuals with Parkinson's Disease would be a useful addition to the currently available treatments for this disabling condition.

2.1 Background

Constipation is common in Parkinson's Disease and often becomes symptomatic years before the onset of the motor dysfunction and the subsequent diagnosis of Parkinson's Disease (Lin et al., 2014; Savica et al., 2009). There is substantial evidence that the neurodegenerative process

associated with Parkinson's Disease, namely the accumulation of toxic aggregates of alpha-synuclein, occurs within the enteric nervous system years before they appear within the brain.

(Braak et al., 2006). It is believed that the enteric nervous system (ENS), with its vast surface area, is subject to continuous insults from infectious agents and toxic substances. Although the function of alpha- synuclein is not known, inflammation within the nervous system leads to an increase in its intracellular levels (Allen Reish and Standaert, 2015). In individuals with Parkinson's Disease the increase in alpha- synuclein leads to the formation of neurotoxic aggregates, perhaps because of a failure by the neuron (due to genetic factors) to effectively dispose of them (Sahay et al., 2016). The aggregates of alpha- synuclein then traffic along the vagal nerve to the dorsal motor nucleus within the brainstem, and from there to more rostral structures (Greene et al, 2014).

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. For many individuals, bowel issues represent a significant detriment to quality of life. Failure to effectively manage this problem can also lead to bowel obstruction, especially as the terminal phase of Parkinson's Disease approaches. A limited number of therapies have been subjected to clinical trials and they include agents that increase the fluid content of the stool, either by blocking fluid resorption or increasing the osmolar load within the intestine (Ondo et al., 2012; Zangaglia et al., 2007).

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). When squalamine is applied to the isolated colon and/or jejunum of a mouse, the compound stimulates organized peristaltic waves (Kunze et al, manuscript in preparation). This effect occurs through direct stimulation by squalamine of the intrinsic primary afferent neurons (IPANS) which are the most abundant type of neuron within the ENS. The IPANs, in turn, excite the ganglia of the myenteric plexus, and promote propulsive peristalsis. In addition, intense electrical signals are directed to the brainstem via the vagus following exposure of the ENS to squalamine which persist for some time. Of relevance to this clinical study, squalamine reverses the impaired motility of the colon from aged animals engineered to express a Parkinson's Disease phenotype.

Furthermore, in a recent study, squalamine has been shown to prevent the formation of toxic alpha- synuclein aggregates (Perni et al., 2017). The molecule accomplishes this as a consequence of its mechanism of action and the mechanism by which alpha-synuclein aggregates to form toxic polymers. Squalamine enters a neuron and binds to the negatively charged phospholipids that are present on the cytoplasmic face of the neuron's plasma membrane, effectively neutralizing the negative charge of the membrane surface. Alpha-synuclein normally binds to the cytoplasmic face of the membrane via the positive charge on its N-terminus. Thus, when squalamine binds to the cytoplasmic face of the plasma membrane it displaces alpha-synuclein into the cytoplasm. As the concentration of alpha-synuclein on the membrane decreases, the likelihood that monomers will aggregate into higher order polymers decreases, thus reducing the rate of formation of aggregates and thereby rescuing neurons from certain death. At the same time, squalamine excites the neuron by displacing certain ion channels and transporters from the membrane. Thus, squalamine both excites the neurons of the ENS and protects them from alpha-synuclein induced cytotoxicity. It is for these reasons that we will evaluate squalamine as a therapeutic to treat the constipation associated with Parkinson's Disease and why we will explore its effect on sleep architecture and hallucinations/delusions.

2.2 Rationale for the Study

Constipation is a major clinical component of Parkinson's Disease and is reported to occur in greater than 60% of affected individuals. The pathophysiological basis of constipation in Parkinson's Disease is generally believed to be due to delayed transit through the colon (Edwards et al., 1991; Klingelhoefer and Reichmann, 2015). Several studies have demonstrated that transit of stool through the colon of an individual with Parkinson's Disease is about 50% that measured in age matched controls. Consequently, both stool frequency and stool consistency are abnormal in Parkinson's Disease. For many patients, as well as those caring for these individuals, constipation remains a significant morbidity associated with the condition (Salat-Foix and Suchowersky, 2012; Sung et al., 2014).

Few placebo-controlled clinical trials have been conducted in the Parkinson's Disease population to assess the efficacy of therapeutics that could be of value. Addition of fiber to the diet, although increasing stool volume, is reported to have no effect on colon transit time (Ondo

et al., 2012; Zangaglia et al., 2007). An osmotic laxative, polyethylene glycol (Magrogol) has been studied in a small placebo controlled clinical trial of individuals with mild constipation, and shown to provide benefit with respect to stool frequency and consistency (Zangaglia et al., 2007). A short term placebo controlled trial of Lubiprostone, a chloride channel activator which increases intestinal fluid secretion, was only effective in about 50% of those treated, and resulted in passage of loose stools/diarrhea in place of constipation (Ondo et al., 2012). Furthermore, Lubiprostone delays gastric emptying (Camilleri et al., 2006), a function already compromised in Parkinson's Disease.

The pathophysiology of the gastrointestinal (GI) dysfunction in Parkinson's Disease involves deposition of alpha-synuclein within both the 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. Numerous studies suggest that the alpha- synuclein aggregate formation begins in the ENS of the Parkinson's Disease individual many years before the onset of the motor symptoms (Braak et al., 2006). As a consequence of the normal retrograde neuronal trafficking that occurs within the vagus nerve, toxic aggregates are transported from the neurons of the ENS to the dorsal motor nucleus of the vagus, and then, gradually to sites within the brain that are involved in physical movement and balance (Greene, 2014; Visanji et al., 2014). Because the constipation is fundamentally of an acquired neurodegenerative nature, it differs, so far as we now understand, from other forms of this condition (Visanji et al., 2014).

The mechanism of action of squalamine has been elucidated over the past decade. The molecule is structurally related to a bile salt, but bears a polyamine, giving it a net positive charge. It is produced naturally in the liver of the dogfish shark and delivered along with bile salts to small intestine via the bile duct. Based on studies conducted ex vivo with isolated segments of mouse jejunum and colon, and our knowledge of squalamine's mechanism of action at a cellular level, we can understand the prokinetic effects of squalamine on intestinal motility. Once within the lumen of the intestine, squalamine is transported from the epithelium into the lamina propria where it enters neurons of the ENS, including the intrinsic primary afferent neurons, the most abundant neuron of the ENS (Furness et al., 1999). Once within the neuron, the positively charged squalamine molecule is attracted to the inner face of the plasma membrane, which has

a negative surface charge, due to the presence of anionic phospholipids, neutralizing the negative charge (Zasloff et al., 2011). As a consequence, many proteins that are bound to the inner face of the cytoplasmic face of the plasma membrane are displaced or functionally altered, including ion channels and transporters (Sumioka et al., 2009; Yeung et al., 2008). By this mechanism, squalamine activates the firing of the IPANs, which in turn, activate the myenteric plexus, resulting in propulsive migrating motor complexes (MMCs), and direct electrical activity rostrally to the brainstem via the vagus (Kunze et al, manuscript in preparation).

Alpha-synuclein normally binds to membranes composed of a high proportion of anionic phospholipids via its positively charged N-terminus (Shi et al., 2015). When the intracellular concentration of alpha-synuclein increases, the concentration of alpha-synuclein molecules bound to the inner face of the cytoplasmic membrane increases proportionally, and as it does the probability that they will physically contact one another and aggregate increases. In individuals with Parkinson's Disease, these aggregates grow in size and eventually overwhelm the cell's capacity to digest the inclusions, whereas in those without Parkinson's Disease these aggregates are cleared by autophagy (Recchia et al., 2004). In a recent study we show that squalamine effectively displaces alpha-synuclein from the surface of a negatively charged membrane surface, and completely prevents aggregate formation. These studies have been conducted in specialized in vitro systems as well as in a validated in vivo model of Parkinson's Disease, a genetically engineered strain of *C. elegans* expressing human alpha-synuclein. Oral treatment of *C. elegans* with squalamine prevents the aggregation of alpha-synuclein within the muscles of the animal and reverses the paralysis caused by these aggregates (Perni et al., 2017).

Thus, squalamine has the remarkable property of stimulating peristaltic activity by directly activating the neurons of the ENS and via the same mechanism, inhibiting the formation of neurotoxic alpha-synuclein aggregates within the same neurons. In principle, squalamine could reverse the neurodegenerative process within the ENS of the individual with Parkinson's Disease. If indeed the pathophysiology of the central nervous system (CNS) component of Parkinson's Disease occurs as a consequence of the trafficking of alpha-synuclein aggregates from the ENS to the brain via the connecting nerves, then squalamine could have an impact on the disease process occurring within the CNS both therapeutically and prophylactically.

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 study consisted of an 8 to 12-week Stage 1 period, and an 8 to 10-week Stage 2 period. All subjects received the study drug during one of the observational periods of the study. The study evaluated the safety of ENT-01, and its effect on bowel function and neurologic symptoms.

In Stage 1, 10 subjects received escalating single doses from 25 mg to 200 mg/day or maximum tolerated dose (MTD). The goal was to determine safety and tolerability. In Stage 2, 34 subjects received daily doses escalating from 75 mg to a maximum of 250 mg/day, a dose that induced change in bowel function or MTD, followed by a "fixed" dose for 4 days, and a 2-week washout. Primary efficacy endpoint was defined as increase of 1 complete spontaneous bowel movement (CSBM)/week, or 3 CSBM/week over 14-day baseline period. Safety was also assessed.

In Stage 2, 82.4% subjects achieved the primary efficacy endpoint. Mean CSBM/week increased from 1.2 at baseline to 3.6 during fixed dose ($p=1.2x10^{-7}$). Common adverse events included nausea in 21/44 (47%) and diarrhea in 18/44 (40%) subjects. No study-drug related serious adverse events were reported. Significant and lasting improvements were observed in MDS-UPDRS scores (p=0.0008), cognition (MMSE) (p=0.0004), hallucinations, sleep, and circadian rhythm (p=0.016). Systemic absorption was < 0.3%.

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.

Phase 2b (ENT-01-030) Dosing Rationale

Dosing will range from 75 mg to 250 mg. The dose will be taken upon awakening on an empty stomach along with 8 oz. of water simultaneously to levodopa (if applicable). The compound is highly charged and will absorb to foodstuffs, so subjects will not be allowed to ingest any food for at least 60 minutes after taking study medication.

Subjects are not allowed to miss a dose, however, in the event this does happen, subjects must wait a minimum of 5 hours post ingestion of a meal and at least 1 hour prior to the next meal to take the missed dose.

In the recently completed Phase 2a study involving subjects with Parkinson's Disease-related constipation, ENT-01 was administered orally both in single-dose (Stage 1: n=10) and multiple daily doses (Stage 2: n=34) and was well tolerated to doses up to 250 mg. Maximal treatment duration in Stage 2 was 25 days. In this 2b study, daily doses of ENT-01 will be administered orally to subjects with Parkinson's Disease-related constipation and maximum treatment duration will also be 25 days.

Humans have long been exposed to low doses of squalamine (milligram to microgram) in the various commercial dogfish shark liver extracts available as nutraceuticals (e.g., Squalamax). In addition, following systemic administration squalamine is cleared by the liver and excreted as the intact molecule (in mice) into the duodenum through the biliary tract. Drug related GI toxicology has not been reported in published clinical trials involving systemic administration of squalamine. ENT-01 has limited bioavailability in rats and dogs. Based on measurement of portal blood concentrations following oral dosing of radioactive ENT-01 to rat's absorption of ENT-01 from the intestine is low. As a consequence, the principal focus of safety is on local effects on the gastrointestinal tract. However, ENT-01 appears to be well tolerated in both rats and dogs.

Because local gastrointestinal intolerance is the primary adverse effect, safety margins based on mg/kg/day of ENT-01 are considered an appropriate safety metric based on allometric comparisons of gastrointestinal dimensions between species (Kararli, 1995; Sharma and McNeill, 2009).

3 OBJECTIVES AND ENDPOINTS

3.1 Primary Objective and Endpoints

The purpose of this study 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.

Safety:

The **primary safety objective** of this study will be the evaluation of the safety of repeated daily oral dose of ENT-01 for up to 25 days in subjects with PD-related constipation.
Safety endpoints will include adverse events as evaluated with subject self-reporting, vital signs, clinical chemistry and hematology blood analyses, urinalysis, and electrocardiogram (ECG).

It is anticipated that no more than 2 subjects out of 60 (3.3%) will have an adverse event 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 adverse event 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 rules will include:

- Reaching dose-limiting tolerability (DLT) before a prokinetic effect
- Having a non-DLT gastrointestinal adverse event > grade 3 within 24 hours of taking study medication or placebo, 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)

Tolerability
The primary tolerability objectives will be evaluation of recurrent vomiting, recurrent diarrhea, abdominal pain and dizziness.

Tolerability endpoints are referred to as the DLT and will include:

- Recurrent vomiting defined as 3-5 episodes of vomiting within 24 hours of taking study medication/placebo
- Recurrent diarrhea defined as 7 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.

Efficacy:
The primary efficacy objective will be evaluation of the efficacy of repeated daily doses of ENT-01 for up to 25 days in improving the CSBM rate.

A CSBM is a bowel movement where the subject indicated that he/she was "done for the day", that he/she had "no more poop left to pass" and the bowel movement was not a1 or 2 on the Bristol stool scale and did not occur within 24 hours after taking rescue medication. For each subject, theFixed Dose period begins on the first day of the subject's prokinetic dose, (or the highest dose at which the subject did not have a DLT), following the incremental dosing algorithm definedin Section 4.2, Visit 2: Randomization (Study Day1).

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. The fixed dose response will be obtained as the weekly CSBM rate during the Fixed Dose period. For each subject, the Fixed Dose period begins on the first day of the subject's prokinetic dose, (or the highest dose at which the subject did not have a DLT), following the incremental dosing algorithm defined in section 4.2 Visit 2: Randomization (Study Day 0, treatment begins the following day at home).

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.

3.2 Secondary Objectives and Endpoints

The secondary objectives of the study are:

1. To determine if the correct starting dose (75 mg or 150 mg) can be predicted from the baseline weekly CSBM rate
2. 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)

Secondary efficacy endpoints will include the following:

- 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 5). 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 6). 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 aCSBM.

- 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.

3.3 Exploratory Objectives and Endpoints

Exploratory objectives of the study are:

- 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, 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.

Exploratory endpoints include:

- 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 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 at Visit 2, and subsequently will only be administered to those subjects with hallucinations and/or delusions on both visits (as determined by a SAPS-PD score >0 at Visit 1 and 2). Subjects with SAPS-PD score >0 at baseline and randomization visits will be included in the analysis.
- Improvement of the symptoms of Parkinson's Disease as assessed by the MDS-MDS-UPDRS: The first 3 parts of the MDS-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 MDS-UPDRS, but total score (Parts 1+2+3) will also be reported. ***The assessment will be performed during the "ON" phase, approximately 60 minutes after taking levodopa***. In individuals who are "on" earlier or later, the UPDRS will be performed when they are "ON".
- 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.

- 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 (20 subjects).

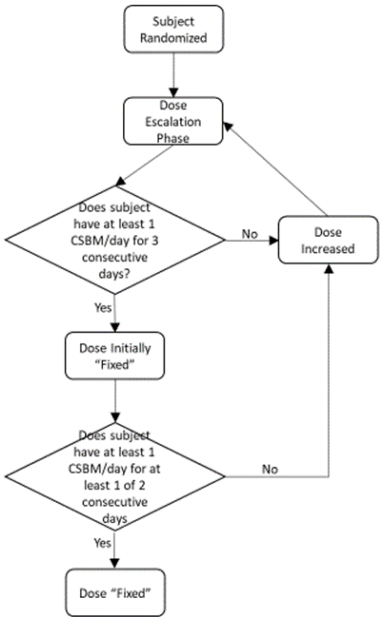
- Change in stool microbiome during treatment: Since ENT-01 has antimicrobial properties in vitro, it may alter the intestinal microbiome; the subject's stool will be analyzed for common intestinal bacteria and viruses at Baseline and Fixed Dose, periods (20 subjects). If subjects discontinue from the study prior to the end of the Fixed Dose period, stool will be collected and analyzed at the Early Termination visit, as applicable.

4 STUDY DESCRIPTION

The first part of this Phase 2b study was conducted as a multicenter, randomized, double-blind, placebo-controlled study. Each subject's total study duration was no more than 10 weeks and total active dosing days were 25 days. 72 subjects were randomized in a 3:1 ratio to active treatment or placebo, respectively. 54 subjects received active treatment and 18 received placebo. Randomization was stratified on baseline weekly CSBM rate. Subjects with baseline CSBM rates of 1.0-3.0/week were randomized to begin with 75 mg of ENT-01 (3 tablets) or 3 placebo tablets: subjects with baseline CSBM rates of 0-0.9/week were randomized to begin with150 mg of ENT-01 (6 tablets) or 6 placebo tablets. The dose was then escalated every 3 days in increments of 25 mg (or 1 placebo tablet) until either an effective dose, a maximum tolerated doseor 250 mg was reached, whichever came first. The dose was then "fixed" at that dose for the remainder of the 25-day treatment period.

In this amendment of the protocol, the study will be conducted as a multicenter, randomized double-blind, placebo-controlled study. Each subject's total study duration will be no more than 10 weeks and total active dosing days will be 25 days. Up to 80 subjects will be randomized across up to 25 U.S. sites to ensure approximately 60 subjects will complete all visits, assuming a 25% early termination rate. Randomization will be 1:1 to active treatment or placebo.

Randomization will be stratified based on baseline weekly CSBM rate. Subjects with baseline CSBM rates of 1.0-3.0/week will be randomized to begin with 75 mg of ENT-01 (3 tablets) or 3 placebo tablets: subjects with baseline CSBM rates of 0-0.9/week will be randomized to begin with 150 mg of ENT-01 (6 tablets) or 6 placebo tablets. The dose will then be escalated every 3 days in increments of 25 mg (or 1 placebo tablet) until either an effective dose, a maximum tolerated dose or 250 mg is reached, whichever comes first. The dose will then be "fixed" at that dose for the remainder of the 25-day treatment period. The dosing logic is illustrated in the diagram below.



- The subject will be randomized to either placebo or ENT-01, with the starting number of tablets based on the baseline CSBM rate.
- The dose will be escalated every 3 days by 1 tablet (25mg ENT-01 or placebo)
- If the subject passes at least 1 CSBM/day on each of 3 consecutive days the dose is "fixed." Otherwise, the dose is escalated.
- If, over the 2 days following the "fixed" dose, the subject passes at least one CSBM, the subject will remain on that dose for the remainder of the 25-day treatment period (until Visit 4).
- If the subject fails to pass a CSBM on at least one of the 2 days following the "fixed" dose, the dose is then escalated by 1 tablet and CSBM rate evaluated as above.
- Dose escalation can continue until either the MTD or the dosing limit of 250 mg (or 10 placebo tablets) is reached.

Each subject will have 6 visits to the clinic: Visit 1/Screening Visit; Visit 2/Randomization Visit; Visit 3/Follow up Visit (during the treatment period); Visit 4/Single-Blind Placebo Withdrawal Period Visit, Visit 5/Washout Period; and Visit 6/ End of Study Visit. It is anticipated that the duration of study participation for each subject will be approximately 10 weeks.

For the duration of the study, subjects will discontinue opiates and all laxatives, bulking agents, softeners and suppositories. They may continue taking proton pump inhibitors and antacids

until the end of the screening period. Throughout the study, subjects may continue antihistamines, anti- cholinergic, anti-psychotic medication, dopamine and anti-depressant medication at stable doses.

Each subject will be randomized to a pre-defined dose based upon their baseline weekly complete spontaneous bowel movements (CSBM) rate, then will be able to increase/adjust their dose every three days. This is called the Dose Adjustment period. After the subject documents three CSBMs in each of three consecutive days, the subject will "fix" at their current dose. Over

the next 2 days, the subject will need to document a CSBM on at least 1 day. If this occurs, the "fixed" dose will continue for the remainder of the 25-day treatment period. If at least 1 CSBM does not occur over the next 2 days, then the subject will escalate the dose by 25mg. This is called the Fixed Dose period. ***In other words, the subject's dose can't be hard "fixed" until a CSBM has occurred on at least 4/5 consecutive days at a given dose.*** The subject's total number of days on active study medication (Treatment Period) cannot exceed 25 days.

Throughout the study, subjects will keep daily electronic stool diaries. The stool diaries will record information including stool frequency, consistency, ease of evacuation, completeness, and the use of rescue medication. Stool related information will be entered immediately upon completing the bowel movement. This information will be collected electronically through web-based questionnaires that the subjects and/or their caregivers complete. Site staff will monitor these entries regularly to ensure that subjects are not at risk of becoming impacted and are using rescue medication as directed. Site staff will contact subjects if the web-based questionnaires are not being completed on a daily basis or if there are any concerns about a subject's safety.

Rescue medication (suppositories and enemas) will be provided to subjects and will be initiated in the evening of the third day if a subject has had no bowel movement for three consecutive days.

Symptoms of Parkinson's Disease will be assessed using the Movement Disorder Society's Unified Parkinson's Disease Rating Scale (MDS-UPDRS) during "ON" states, approximately one hour after levodopa intake (if applicable).

If the "ON" time is not 60 minutes after L-Dopa, assessment has to occur at "ON" time or whenever the subject is "ON".

Subjects who prematurely discontinue study drug for any reason will be asked to have an Early Termination (ET) visit within 3 days following the last study drug dose. If a subject discontinues study drug during a scheduled visit, then the Early Termination visit should be conducted at that time. These subjects will also be asked to complete the Wash-Out period and return for the End of Study (EoS) Visit.

Study (EoS) Visit. The stool sample (if applicable) should be collected during the ET visit if the subject does not wish to return for the EoS Visit.

Figure 1: Study Design

Visit Schedule Overview					
Visit 1	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**
Screening	Randomization and Drug Dispensation	Follow up	End of Treatment – Beginning of Single Blind Placebo Withdrawal	End of Single blind Placebo – Beginning of Wash Out	End of Study
Day -21	Day 1 +/- 8days	Day 10 +/- 3 day	Day 26 - 2 days	Day 39 +/- 8 days	Day 69 +/- 8 days

Commented [A2]: Should this be +8days or +/- 8days

4.1 Visit 1: Screening Visit (Study days -21 to -1)

The purpose of the baseline or "screening" period is to establish constipation severity off laxatives. At the end of the screening period the subject's eligibility will be assessed by an

Evaluation Committee.

During Visit 1, the following assessments and procedures will be performed:

- Informed consent
- Inclusion/Exclusion Criteria
- Demographics/Medical History
- Complete Physical Exam
- Height
- Weight
- Orthostatic Vital Signs – 3 sets: Vital signs will include supine blood pressure and heart rate, sitting or standing blood pressure and heart rate, respiratory rate, and body temperature. Sitting or standing measurements will be taken approximately 5 minutes after the subject has been sitting or standing. The sets should be taken throughout the Visit and not sequentially.
- 12-lead ECG
- Labs and urinalysis: as defined in APPENDIX 1.
- Pregnancy Testing – For women of child-bearing potential, this is done on-site with the urine pregnancy kit (UPT) provided. If the on-site UPT is positive, then a serum pregnancy test will be done. For post-menopausal women less than 60 years or age, a serum FSH will be done at the screening visit only.
- Instructions to Discontinue Laxatives and other Constipation medications. All prescription laxatives (Linzess, Amitiza), over the counter laxatives (Miralax, Ex-lax, Bisacodyl/Dulcolax), suppositories (Bisacodyl/Dulcolax), enemas (Fleets), and dietary supplements to increase bowel movements (chia seeds, bran, fiber supplements) will be discontinued.
- Dispense Rescue Medication: Subjects will be provided with suppositories and enemas. Anytime during the study if a subject does not have any bowel movement for three consecutive days, rescue medication should be used to evacuate their bowel as directed by the

site. In the rare event that the above does not produce a bowel movement, the subject will be instructed to notify the site and ask for direction.

- Stool and laxative Diary Instructions: Subjects will be taught how to use the I-Pad for stool and laxative diaries. They will be instructed to take the I-Pad to the toilet and fill it in immediately after a bowel movement. They will be taught the definition of a CSBM. If the subject "feels like he/she is completely emptied out" and that "they have no more stool left to pass", and the stool is not just a few pebbles, then that will be a CSBM (a complete BM). If the subject leaves the house for a few hours, they will be told to take the I-Pad with them in case they have a BM elsewhere. Each BM will be entered immediately. If no entries have been made for the day, the subject will be sent 3 reminders starting at 7pm. Laxative use will also be entered immediately into the laxative diary. Multiple uses will be entered multiple times.
- AE Review
- Prior and Concomitant Medication
- Rome IV questionnaire
- MDS-UPDRS – The MDS-UPDRS should be administered while the subject is in an ON state, approximately one hour after taking levodopa (if applicable). If the "on" time is earlier or later than 60 minutes after L-Dopa, UPDRS will be performed at that time.
- Subjects will be invited to participate in two sub-studies, consisting of obtaining levodopa PK draws pre-dose, and 20, 40 and 60 minutes post-dose at Visits 2 and 3, 4, 5 and 6 and (2) stool sample collection on Visits 2 and 4. This first twenty subjects to affirm consent will participate in the L-Dopa sub-study and the first 20 subjects to affirm consentwill participate in the stool microbiome study.
- Questionnaires:
    - C-SSRS
    - SAPS-PD and hallucination interview (only those subjects who have a SAPS-PD score > 0 at the Screening Visit and Randomization Visit).
    - BDI-II
    - MMSE

- Dispense Stool Collection Kit – Subjects participating in the sub-study will be given astool collection kit so they can bring a stool sample at Visit 2

For subjects who score > 0 on the SAPS-PD at Visit 1 and Visit 2, the SAPS-PD and hallucination interview are repeated at Visit 2.

4.2 Visit 2: Randomization (Study Day 1 + 8 days)

The purpose of this period is to establish the prokinetic dose for subjects randomized to treatment (or placebo). The prokinetic dose is the dose that results in a CSBM on all three dosing days at any given dose. Subjects will also be required to have a CSBM on at least 1 of 2 subsequent days at the "fixed" dose. In other words, ***the dose will only be "fixed" for the remainder of the treatment period if the subject has CSBMs on at least 4 of 5 consecutive days at a given dose***.

In this amended study, subjects will be randomized in a 1:1 ratio to receive either ENT-01 or placebo depending on baseline weekly CSBM rate:

- Subjects with baseline CSBMs of 0-0.9 will be randomized to begin treatment with 150 mg of ENT-01 or 6 tablets of placebo.
- Subjects with baseline CSBMs of 1.0-3.0 will be randomized to begin with 75 mg of ENT-01 or 3 tablets of placebo.

Subjects will self-administer the study medication at home, beginning the day after Visit 2. The dose will be taken upon awakening on an empty stomach along with 8 oz. of water. The subject will be instructed to remain in the upright position for the next 60 minutes. This can be in the sitting or standing position, and the subject should be encouraged to walk around, go to the kitchen and make a cup of tea or coffee (with or without milk or sugar), to watch the news or read a paper in the upright position. The subject will also be instructed to not eat for at least 60 minutes thereafter.

After the first dose, the subject will be advised to stay home for 4-6 hours in case an emergency evacuation occurs. The subject can determine what to do after subsequent doses. Subjects are not allowed to miss a dose, however, in the event this does happen, subjects must wait a minimum of 5 hours post ingestion of a meal and at least 1 hour prior to the next meal to take the missed dose.

Any given dose is repeated for 3 consecutive days. If the subject has not produced a CSBM

within 24 hours of a dose on all three days at the initial dose, then they will increase their daily dose by 1 tablet (i.e., 25 mg for active treatment), repeating that dose for three consecutive days. This 3-day incremental dosing will continue until the subject:

- Reaches the prokinetic dose. The prokinetic dose is determined when a subject has entered CSBMs on at least 4 of 5 consecutive days at a given dose. The subject then remains on that dose until Visit 4;
- Reaches the maximum dose of 10 tablets (i.e., 250 mg for active treatment); or,
- Reaches the dose limiting tolerability (DLT).

If the subject has 4 or more episodes of diarrhea with a consistency of 6-7 (see APPENDIX 6) within 24 hours of a dose on 2 consecutive days at a given dose, the dose will be reduced by 25 mg and fixed at that dose assuming diarrhea subsides.

Should a prokinetic response not occur at 250 mg (or 10 tablets), subjects will continue to take10 tablets daily for the remainder of the 25-day treatment period.

During this visit, the following assessments and procedures will be performed:

- Review of Inclusion/Exclusion Criteria
- Confirmation of eligibility: To be randomized to receive double-blind study medication in this study, subjects must meet the following criteria:
- Have at least 11 'Available Data Days' (days with e-Diary compliance sufficient for determining whether a CSBM occurred) (Subjects with fewer than 11 diary days will continue to fill in their diaries until 11 days' worth of information is available and randomization will be delayed). Have had fewer than 3 complete spontaneous bowel movements per week based upon the average CSBM rate reported during the 2-week Screening Period. This rate will becalculated centrally with a computer-based system and requested by the site the day before randomization.
- Be deemed eligible for randomization by the enrollment committee
- Weight
- Orthostatic Vital Signs
- 12-lead ECG

- Pregnancy Testing – For women of child-bearing potential only.
- Randomization: Sites will utilize a centralized, computer-based system (IWRS) to obtain the randomization bottle (s) assigned to each subject.
- In-clinic Levodopa Dosing – Subjects will take their morning dose of levodopa in the clinic.
- Subjects will be given study medication and will take their first dose the following day at home, on an empty stomach. The day after Visit 2 will be the first day of treatment.
- Levodopa PK Draws – The first daily dose of L-Dopa will be administered in clinic. Blood samples will be obtained pre-L-Dopa dose and at 20, 40-and 60-minutes post-dose. (Applicable to the levodopa PK sub-study only.)
- Dispense Rescue Medication, as needed. Subjects continue appropriate use of rescue medication, ensuring adequate bowel movements every 3 days.
- AE Review
- Prior and Concomitant Meds
- C-SSRS
- SAPS-PD (if SAPS-PD score is greater than 0 on Visit 1 and 2, subject will have SAPS-PD and hallucination questionnaire administered at each subsequent visit).
- PAC-SYM
- PAC-QOL
- Collect Stool Sample (if participating in stool sub-study)
- Dispense IP/placebo and instruct subjects to take their second dose of study drug the following morning. Subjects will be advised to stay at home for 4-6 hours in case they need to evacuate urgently.

4.3 Visit 3: Study Day 11 (+/- 3 days)

The purpose of this visit is to evaluate the incidence of adverse events and to review the subject's dosing experience by reviewing ePRO entries with the subject.

During this visit, the following assessments and procedures will be performed:

- Brief Physical Exam
- Weight
- Orthostatic Vital Signs
- 12-lead ECG
- Labs and urinalysis as defined in APPENDIX 1.
- Pregnancy Testing - For women of child-bearing potential only.
- For subjects participating in the L-Dopa sub-study, subjects will be instructed to come to clinic as early as possible and on an empty stomach. They will first take their study medication (ENT-01 or placebo) in clinic. Thirty minutes after the ENT-01/placebo dose, subjects will take their morning dose of levodopa.
- Levodopa PK Draws - Blood samples will be obtained before the L-Dopa dose and at 20, 40 and 60 minutes after the dose.
- IP/Placebo Drug Accountability and Dispensation
- Dispense Rescue Medication, as needed. Subjects continue appropriate use of rescue medication, ensuring adequate bowel movements every 3 days.
- Stool Diary Review
- AE Review
- Prior and Concomitant Meds
- MDS-UPDRS - The MDS-UPDRS should be administered while the subject is in an ON state, approximately one hour after taking levodopa (if applicable), or whenever the "ON" state occurs.
- Questionnaires:
    - C-SSRS
    - BDI-II
    - MMSE

- PAC-SYM
- PAC-QOL
- SAPS-PD and hallucination questionnaire (if applicable)

- Dispense Stool Collection Kit - Subjects participating in this sub-study will be given a stool collection kit so they can bring a stool sample at Visit 4.

4.4 Visit 4: End of treatment period/ Beginning of Placebo Period (Study Day 26)(- 2 days)

The purpose of the treatment period is to establish the impact of ENT-01 on CSBM rates. The purpose of the placebo period is to determine the duration of the treatment effect once study medication is discontinued. At this visit, all subjects will be given placebo and will continue taking the same number of tablets as they were taking at the maximum "fixed" dose period. The subject will not be made aware that the study medication provided is a placebo.

During this visit, the following assessments and procedures will be performed:

- Brief Physical Exam
- Weight
- Orthostatic Vital Signs – 3 sets: See screening description
- 12-lead ECG
- Labs and urinalysis as defined in APPENDIX 1.
- Pregnancy Testing - For women of child-bearing potential only.
- IP/Placebo Collection and Accountability
- Single-blind Placebo Dispensation
- For subjects participating in the L-Dopa sub-study, subjects will be instructed to come to clinic as early as possible and on an empty stomach. They will first take their study medication (ENT-01 or placebo) in clinic. Thirty minutes after the ENT-01/placebo dose, subjects will take their morning dose of levodopa.
- Levodopa PK Draws - Blood samples will be obtained before the -L-Dopa dose and at 20,

40 and 60 minutes after the dose

- IP/Placebo Collection and Accountability
- Dispense Rescue Medication, as needed. Subjects continue appropriate use of rescue medication, ensuring adequate bowel movements every 3 days.
- Stool Diary Review
- AE Review
- Prior and Concomitant Meds
- MDS-UPDRS - The MDS-UPDRS should be administered while the subject is in an ON state, approximately one hour after taking levodopa (if applicable), or whenever "ON" occurs.
- Questionnaires:
    - C-SSRS
    - BDI-II
    - MMSE
    - PAC-SYM
    - PAC-QOL
    - SAPS-PD and hallucination questionnaire (if applicable)
- Collect Stool Sample (if applicable)

4.5 Visit 5: End of placebo period/ beginning of Wash-Out (Study Day 39) (+/- 8 days)

The purpose of the Wash-out period is to determine the duration of treatment effect. Subjects will be taken off placebo (study medication). They will continue to keep daily stool diaries for the duration of this period. During the first 3 weeks, subjects will continue to use rescue medication as needed. The following day, i.e., day 22 of the Wash-Out Period, subjects will be instructed to restart all their original medications, including oral laxatives.

During this visit, the following assessments and procedures will be performed:

- Brief Physical Exam
- Weight
- Orthostatic Vital Signs
- 12-lead ECG
- Labs and urinalysis as defined in APPENDIX 1.
- For subjects participating in the L-Dopa sub-study, subjects will be instructed to come to clinic as early as possible and on an empty stomach. They will first take their study medication (placebo) in clinic. Thirty minutes after the placebo dose, subjects will take their morning dose of levodopa.
- Levodopa PK Draws - Blood samples will be obtained before the -L-Dopa dose and at 20, 40 and 60 minutes after the dose.
- Pregnancy Testing - For women of child-bearing potential only.
- Dispense Rescue Medication, as needed. Subjects continue appropriate use of rescue medication, ensuring adequate bowel movements every 3 days.
- IP/Placebo Collection and Accountability
- Stool Diary Review
- AE Review
- Prior and Concomitant Meds
- MDS-UPDRS - The MDS-UPDRS should be administered while the subject is in an ON state, approximately one hour after taking levodopa (if applicable), or whenever “ON” occurs.
- Questionnaires:
    - C-SSRS
    - BDI-II

- MMSE
- PAC-SYM
- PAC-QOL
- SAPS-PD and hallucination questionnaire (if applicable)

4.6 Visit 6: End of Study Visit (Study Day 69) (+/- 8 days)

This is the final visit of the trial. During this visit, the following assessments and procedures will be performed:

- Brief Physical Exam
- Weight
- Orthostatic Vital Signs
- 12-lead ECG
- Labs and urinalysis as defined in APPENDIX 1
- Pregnancy Testing - For women of child-bearing potential only.
- In-clinic Study Medication and Levodopa Dosing - Subjects will be instructed to come to clinic as early as possible. They will take their first morning dose of levodopa in the clinic.
- Levodopa PK Draws - Blood samples will be obtained before the -L-Dopa dose and at 20, 40 and 60 minutes after the dose.
- IP/Placebo Drug Accountability
- Stool Diary Review
- AE Review
- Prior and Con Meds
- MDS-UPDRS - The MDS-UPDRS should be administered while the subject is in an ON state, approximately one hour after taking levodopa (if applicable), or whenever “ON” occurs.

- Questionnaires:
    - C-SSRS
    - BDI-II
    - MMSE
    - PAC-SYM
    - PAC-QOL
    - SAPS-PD and hallucination questionnaire (if applicable)

4.7 Early Termination Visit (if appropriate)

If a subject terminates the study prematurely for any reason, the following procedures will be performed:

- Complete physical examination, including weight
- Orthostatic vital signs
- 12-lead ECG
- Laboratory studies and urinalysis
- Blood draw for L-Dopa PK sub-study only. Blood samples will be obtained pre-dose and at 20, 40, and 60 minutes post-dose. (Applicable to the L-Dopa PK sub-study only. Subjects will take their dose of ENT-01/matching placebo 30 minutes prior to taking L-Dopa.
- Pregnancy testing, for women of child-bearing potential only.
- IP/Placebo collection and accountability
- Electronic stool diary review
- Review of AEs
- Prior and concomitant medications
- MDS-UPDRS should be administered while the subject is in an ON state, approximately one hour after taking L-Dopa (if applicable)
- Subjects will complete the following questionnaires:
- BDI-II

- MMSE
- PAC-SYM
- PAC-QoL
- If subjects discontinue from the study prior to Visit 4, collect Stool Sample (as applicable)

5 STUDY POPULATION

Subjects will have Parkinson's Disease and constipation as defined by the inclusion criteria. Parkinson's Disease will be diagnosed according to the UK Parkinson's Disease Society Brain Bank criteria.

5.1 Number of Subjects in this amendment

Approximately 80 subjects will be randomized to ensure approximately 60 subjects will complete all visits, assuming a 25% early termination rate.

5.2 Selection Criteria

Subjects will be screened for the presence of constipation at baseline according to the Rome IV criteria and other inclusion criteria below. Onset of constipation before or after a diagnosis of Parkinson's Disease will specifically be noted and its duration and severity established. The daily electronic stool diary will be analyzed at the end of the two-week screening period (Visit 2) to establish the presence of constipation. Subjects with less than three CSBMs per week, without the use of rescue medication, will be randomized during Visit 2.

5.3 Inclusion Criteria

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

1. Subjects aged 30-90 years, both genders
2. Subjects must provide written informed consent and be willing and able to comply with study procedures.
3. Subjects must be diagnosed with Parkinson's Disease defined as the presence of at least three of the following cardinal features, in the absence of alternative explanations or atypical features: rest tremor, rigidity, bradykinesia and/or akinesia, postural and gait abnormalities.
4. There are insufficient criteria for Irritable Bowel Syndrome (IBS)

5. Constipation which has been present for approximately 6 months
6. Body mass index (BMI) of 18-40 kg/m^2
7. Subjects must fulfill Rome IV criteria for functional constipation which includes 2 or more of the following:
    a. Straining during at least 25% of defecations
    b. Lumpy or hard stools in at least 25% of defecations
    c. Sensation of incomplete evacuation for at least 25% of defecations
    d. Sensation of anorectal obstruction/blockage for at least 25% of defecations
    e. Manual maneuvers to facilitate at least 25% of defecations (e.g., digital evacuation, support of the pelvic floor)
8. Self-report of 3 or fewer complete spontaneous bowel movements (CSBMs) per week
9. Loose stools are rarely present without the use of laxatives
10. Subjects must be able to read, understand, and accurately record data into the diary to guarantee full participation in the study.
11. Female subjects must have negative serum or urine pregnancy tests and must not be lactating. For females able to bear children, hormonal (i.e., oral, implantable, or injectable), abstinence, same sex relationships, single-barrier method, or a double-barrier method of birth control must be used throughout the study. A vasectomized partner will be allowed as one in conjunction with another single-barrier method.
12. Female subjects unable to bear children must have this documented in the CRF (i.e., tubal ligation, hysterectomy, or postmenopausal [defined as a minimum of one year since the last menstrual period]). Post-menopausal status will be confirmed by follicle stimulating hormone (FSH) in women less than 60 years of age

5.4 Exclusion Criteria

1. Unable or unwilling to provide informed consent or to comply with study procedures.
2. Diagnosis of secondary constipation beyond that of Parkinson's Disease

3. Review of Screening Diaries indicates either of the following:
    - Fewer than 11 days of diary completion and/or
    - More than 3 complete spontaneous bowel movements per week based upon the average CSBM rate reported during the Screening Period.
4. A compromised gastrointestinal system which includes:
    - Structural, metabolic, or functional GI diseases or disorders
    - Acute GI illness within 2 weeks of the screening visit
    - History of major GI surgery within 30 days of the screening visit (a history of cholecystectomy, polypectomy, hernia repair or appendicectomy are not exclusionary as long as they were performed more than 30 days before the screening visit)
5. Unable or unwilling to withdraw from laxatives, opiates, clonazepam, or any medications which may cause constipation, 2 weeks prior to the dose adjustment period and throughout the rest of the study.
6. Unable or unwilling to withdraw from proton pump inhibitors and antacids at the end of the screening period.
7. Any clinically significant abnormalities on screening laboratories or physical examination requiring further evaluation or treatment.
8. Neurological disorder other than Parkinson's Disease that in the opinion of the investigator might interfere with the conduct of the study.
9. On treatment with intra-jejunal dopamine or carbidopa/levodopa (i.e. Duopa).
10. Subjects starting a new Parkinson's Disease medication or modifying an existing medication within 2 weeks prior to enrollment.
11. Unable to maintain a stable diet regimen.
12. Subjects with a cognitive impairment that preclude them from understanding the informed consent.
13. Subjects placed under legal guardianship.

14. Females who are pregnant or breastfeeding.
15. History of excessive alcohol use or substance abuse.
16. Participation in an investigational drug trial within the month prior to dosing in the present study.
17. Any other reason, which, in the opinion of the investigator, would confound proper interpretation of the study.

5.5 Screen Failures

Screen failures are defined as subjects who consent to participate in the study but are not subsequently randomized to the study drug. Minimal information collected will include informed consent date, baseline subject characteristics, all the eligibility criteria violated, reasons for screen failure, AEs that led to screen failure, and any SAEs; these will be entered in the eCRF.

Subjects who do not meet the criteria for participation in the study (screen failure) may / may not be re-screened.

Any subjects who are re-screened should be assigned the same subject number given at the initial screening.

5.6 Discontinuation Criteria and Early Termination Procedures

Subjects may withdraw voluntarily from participation in the study at any time and for any reason. Subjects may also be withdrawn based on the Investigator's clinical judgment.

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

When a subject withdraws or is withdrawn before completing the study, the date and reason for withdrawal are to be documented. Subjects who withdraw or who are withdrawn prematurely are to attend an early discontinuation visit, at which time they will complete all assessments as outlined in the Schedule of Events (APPENDIX 2).

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

5.7 Concomitant and Prohibited Medication

- Preventive laxatives will be discontinued during the screening period. This will include all prescription laxatives, over the counter laxatives and dietary supplements. Throughout the study, subjects will be allowed to take constipation rescue medication on demand, and only if they have not had a bowel movement in three consecutive days. Rescue medications will be recorded in the electronic daily diary every time a laxative is used. Bowel movement produced within 24 hours of rescue medication or accidental self-prescribed laxative use will not be counted as either spontaneous bowel movement or complete spontaneous bowel movement.
- Proton pump inhibitors and antacids will be discontinued at the end of the screening period and reinstated during the wash-out period.
- Barbiturates, and opiates will be discontinued at the end of the screening period and 2-3 days before dosing and reinstated during the wash-out period. All medications shall be reviewed and dis/approved by the investigator on a case-by-case basis.
- Selective serotonin reuptake inhibitor (SSRI), SNRI, tricyclic antidepressant, anti-cholinergic, anti-histaminergic and anti-psychotic agents are permissible at stable doses.
- Dopamine agonists and amantadine will be allowed if on a stable dose for at least 2 weeks preceding the study.
- Deep brain stimulation is allowed if present for more than 6 months and at stable stimulation parameters.

Subgroup Analyses

Subjects will be analyzed overall by their randomized treatment assignment (i.e., active versus placebo). Subgroup analyses will also be performed by a subject's baseline CSBM rate (i.e., 0-0.9, or 1.0-3.0). The primary efficacy endpoint and key secondary, exploratory and safety endpoints will be summarized by these subgroups. Additional subgroup analyses may be defined in the statistical analysis plan (SAP).

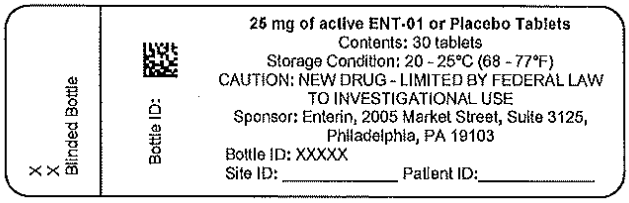
6.0 MATERIALS

6.1 Study Drug

All study medication and placebo will be supplied by Enterin, Inc. as 25 mg tablets packaged in white opaque high-density polyethylene (HDPE) bottles with child-resistant closures.

6.2 Packaging and Labeling

Study medication will be shipped to the investigational sites in blinded packaging.



6.3 Storage, Dispensing and Reconciliation of Study Drug and Identity of Investigational Products

All study medication is to be stored at "Controlled room temperature" as defined below.

Controlled room temperature: The temperature maintained thermostatically that encompasses the usual and customary working environment of 20°–25° (68°–77° F). The following conditions also apply. Mean kinetic temperature not to exceed 25°. Excursions between 15° and 30° (59° and 86° F) that are experienced in pharmacies, hospitals, and warehouses, and during shipping are allowed. Provided the mean kinetic temperature does not exceed 25°, transient spikes up to 40° are permitted as long as they do not exceed 24 h. Spikes above 40° may be permitted only if the manufacturer so instructs.

Articles may be labeled for storage at "controlled room temperature" or at "20°–25°", or other wording based on the same mean kinetic temperature [see also Good Storage and Distribution Practices for Drug Products ⟨1079⟩, Quality Management System, Environmental Management System, Mean Kinetic Temperature (MKT) Calculation].

An article for which storage at Controlled room temperature is directed may, alternatively, be stored and shipped in a cool place or refrigerated, unless otherwise specified in the individual monograph or on the label.

Storage on- site should be in a locked and secure location accessible only to site staff involved with this study. A temperature log must be kept to document temperature which should be recorded at least once each working day. If the temperature is not maintained, the sponsor should be contacted.

If a site becomes aware that study medication has not been properly handled, the sponsor must be contacted immediately. In such an event, study medication should not be dispensed to any subject until the sponsor provides further direction.

Neither the investigator nor any study personnel will distribute any study medication to any person not participating in this study. The study medication will be dispensed at the discretion and direction of the investigator in accordance with the conditions specified in this protocol. It

Commented [A3]: Remaining comment from Carmen to be addressed: Do you want to add the requirement to use min/max temperature measuring devise?

We will discuss at Dev team tomorrow

is the investigator's responsibility to ensure that accurate records of study medication issuance and return are maintained.

Enterin is responsible for the tracking and accountability of study medication dispensed to sites and will inform sites how to return or destroy study medication once it is no longer needed at the site.

Table 6.1: Identity of Investigational Products

Active Dosage form	25 mg Tablet	White Tablet
Placebo Dosage form	25 mg tablet	White tablet Identical in appearance but contains no active ingredient

Table 6.2: Route of Administration

Route/dosage	Oral (*PO*). Doses will require multiple tablets: 75 mg=3 tablets 100 mg=4 tablets 125 mg=5 tablets 150 mg=6 tablets 175 mg=7 tablets 200 mg=8 tablets 225 mg=9 tablets 250 mg=10 tablets
Dosing Instructions	Take 60 min before breakfast with 8 oz. water

7 WARNINGS AND PRECAUTIONS

Subjects will be advised to stay at home for 4-6 hours after taking the first at-home dose of medication in case they need to evacuate urgently. Beyond the first dose, subjects themselves will be in a position to know if and when to leave the house.

8 STUDY PROCEDURES

8.0 Observations and Measurements

Subject informed consent must be obtained prior to conducting any study-related procedures. The Principal Investigator at each center will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the

information provided. All assessments and procedures will be completed according to the Schedule of Events.

8.1 Instructions to Subjects

Subjects and their spouse/companion will be taught how to fill in the daily diaries for stool and laxative. They will be instructed about the use of rescue medication and told to record it in the diary immediately.

8.2 NCI CTC AEv4.03 Definitions of Dose Limiting Adverse Events

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Diarrhea (watery stool)	Increase of <4 stools a day over baseline	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self-care ADL	Life threatening complications ; urgent intervention indicated	Death
Vomiting	1-2 distinct episodesper 24 hrs	3-5 distinct episodes per 24 hrs	6 or more distinct episodes in 24 hrs; tube feeding,TPN or hospitalization indicated	Life-threatening consequences ; urgent intervention indicated	Death
Abdominal pain	Mild pain	Moderate pain; limiting instrumental[1] ADL	Severe pain; limiting self-care ADL[2]	-	-
Postural hypotension[3]	Mild, transient lightheadedness or dizziness upon rising from	Moderate lightheadedness, dizziness or fainting upon	Severe lightheadedness, dizziness or fainting upon rising from	Life-threatening and urgent intervention indicated	Death

	lying or sitting position that does not interfere with ADL	rising from lying or sitting position that limits instrumental ADL; Non-urgent medical intervention indicated	lying or sitting position that limits self-care ADL; Medical intervention or hospitalization indicated		

[1]Preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

[2]Bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden

[3]Definition adapted from combining CTCAE definitions for Hypotension and Dizziness

8.3 Criteria for Dose Limiting Toxicity (DLT) Endpoints:

Recurrent vomiting: 3-5 episodes of vomiting within 24 hours of taking study medication/placebo (Grade 2 adverse event)

Recurrent diarrhea: >7 episodes of diarrhea within 24 hours of taking study medication/placebo (Grade 3 adverse event)

Abdominal pain: Moderate pain that limits instrumental activities of daily living within 24 hours of taking study medication/placebo (Grade 2 adverse event)

Dizziness: Moderate lightheadedness or fainting upon rising from lying to sitting or standing and severe enough to require medical intervention within 24 hours of taking study medication/placebo (Grade 2 adverse event) or either a systolic blood pressure less than 80mmHg or diastolic blood pressure less than 40mm Hg.

8.4 Emergency Unblinding

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 Interactive voice/web-response system (IVRS/IWRS). 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.

8.5 Pre-Existing Medical Conditions

All subjects enrolled in the study will have Parkinson's Disease. Specific pre-existing medical conditions that will be excluded are listed in subject exclusion criteria.

8.6 Treatment Emergent 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.

An adverse event is typically collected after signing the informed consent form and could be related or unrelated to the study drug. A TEAE is for after the subject actually takes the study drug.

Adverse events may be called "baseline-emergent adverse event" which is defined as any event which occurs or worsens during the staged screening process (after informed consent) Visit 2. We will include separate summaries for adverse events that occur prior to the initiation of the treatment and adverse events that occur after the initiation of the treatment (i.e., summary of treatment emergent adverse events).

8.7 Laboratory Abnormalities

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

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

- NCS: Not clinically significant

- LE: Laboratory Error
- PT: Subject abnormal; relates to the subject's usual state of health
- CS: Clinically Significant. This value cannot be explained by any of the other flags.

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

If a laboratory value is considered to be serious and life-threatening the subject should be immediately discontinued from the study and appropriate therapy started.

8.8 Adverse Event Assessment and Recording

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

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

The investigator will use the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) V.4.03 definitions to grade the severity of the event.

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

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

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

Grade 4: Life-threatening consequences; urgent intervention indicated

Grade 5: Death

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

- **Not related:** Evidence indicates no plausible direct relationship to the study medication
- **Remote:** Suggests other conditions are reasonably likely to account for the event including concurrent illness, progression or expression of the disease state, or reaction to concurrent medication
- **Possible:** Suggests that the association of the event with the study medication is unknown; however, the adverse event is not reasonably supported by other conditions
- **Probable:** Suggests that a reasonable temporal sequence of the event with medication administration exists and, based upon the investigator's clinical experience, the association of the event with study medication seems likely
- **Definite:** Suggests that based upon the investigator's experience, the association of the event with the study medication seems very certain.

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

8.9 Reporting Requirements

Any adverse event, including both observed or volunteered problems, complaints, or symptoms that begins any time between the obtaining of informed consent and within 30 days after the

end of the last dose are to be recorded briefly on the appropriate CRF and in detail in the source documents. A check list of adverse events may not be used during this study.

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

Adverse Event: Any untoward medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can be any unfavorable sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational drug, whether or not considered related to the investigational drug.

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

- results in death
- is life-threatening
- requires in-patient hospitalization
- prolongs existing hospitalization
- results in permanent or significant disability or incapacity
- is a congenital anomaly/birth defect
- requires intervention to prevent permanent impairment/damage
- other serious (Important Medical Events) that does not fit the other outcomes, but the event may jeopardize the subject and may require medical or surgical intervention (treatment) to prevent on of the other outcomes.

Life-threatening adverse event: At the time of the event, a subject was at risk of death.
There is a distinction between the severity and the seriousness of an adverse event. Severe is a measurement of intensity, thus a severe reaction is not necessarily a serious adverse event. For example, a headache may be severe in intensity, but would not be serious unless it met one of the criteria for serious adverse events listed previously.

8.10 Serious Adverse Events

Adverse events (AEs) and serious adverse events (SAEs) will be collected from signing of the informed consent until the follow-up contact. The investigator or site staff will be responsible for the detection and documentation of events meeting the criteria and definition of an adverse event or serious adverse event, as provided in the study protocol.

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

In the event of an AE or SAE, it will be the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event and attempt to establish a diagnosis of the event based on signs, symptoms, and other clinical information. The diagnosis was to be documented as the adverse event or serious adverse event and not the individual's signs/symptoms. Once an Investigator becomes aware that a SAE has occurred in a study subject, they are to report the information to Enterin within 24hrs and provide an assessment of causality.

8.11 Notification of Serious Adverse Events

Under IND regulations, 21 CFR Part 312.64, investigators are required to notify the Sponsor/representative immediately via a designated telephone number, within 24 hours of the sites' notification of any SAEs, whether or not considered drug related.

The Sponsor/representative will be notified and in turn, will report serious adverse events to regulatory agencies as required. In addition to the serious adverse events, other events that in the investigator's opinion suggest a significant hazard, contraindication, or precaution should be considered serious. This includes, but is not limited to, blood dyscrasias, endocrine disturbances, hemorrhage from any site, or severe skin disorder. Additional examples are

intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, and development of drug dependency or drug abuse. Hospitalization for elective surgery is not considered an SAE. In addition, pregnancy is not an SAE, but is reportable and must be reported per IND regulations and timelines, 21 CFR Part 312.64, on the SAE reporting form and submitted to the sponsor/representative.

Subjects who experience an SAE must be given appropriate examinations and treatment. The investigator must provide written information to the sponsor/representative as soon as possible.

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

8.12 Reporting a Serious Adverse Event

Any SAE, including death due to any cause that occurs during this study, whether or not believed to be related to the study medication, must be reported immediately (within one business day).
The phone number the sites will use for reporting SAEs is shown below.

NorthAmerica_Medical@parexel.com
Safety Fax: 781-434-5957
Safety Line: 781-434-5010 (Used for any reporting questions that may arise from the site

Specific medical questions can be addressed to the Chief Medical Officer Denise Barbut, MD, FRCP d.barbut@enterininc.com Phone: 917-975-1377

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

All reportable SAEs will be reported to regulatory authorities by the Sponsor/representative according to the required timelines. A copy of all FDA reportable serious adverse events will be

mailed to all investigators participating in ongoing clinical studies with the study medication in order to permit prompt notification of the appropriate institutional review board (IRB).

8.13 Departure from Protocol for Emergency or Adverse Event

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

8.14 Safety and Quality Monitoring

A Data Safety and Monitoring Board (DSMB) has been established to monitor the safety of the subjects during the course of the study. The DSMB (with the requisite Charter) includes members with relevant clinical expertise, including a good understanding of the safety of medications for Parkinson's Disease. These members include a statistician, a gastroenterologist, and a neurologist. The DSMB will be called upon if and when necessary. In addition, subject appropriateness will be monitored by a committee consisting of neurologists, including the national PI and the CMO. The committee will review subject information half-way through the screening period and make recommendations about proceeding to randomization.

8.15 Stopping Rules

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:

- 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)

8.16 Follow-Up and Final Reports

The investigator shall provide Enterin, Inc. with an accurate final report within 1 month after completion, termination or discontinuation of the study. The final report may not precede retrieval of CRFs which have not been monitored.

8.17 Regulatory Aspects

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

9 DATA MANAGEMENT AND STATISTICS

9.0 Populations for Analyses

The following populations will be considered for statistical analyses.

- **Safety Population:** The Safety Population will consist of all subjects who receive at least one dose of study medication during the study.
- **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.
- **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.
- **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.

9.1 Statistical Analyses

A separate statistical analysis plan (SAP) with additional details on the analyses to be performed and analysis methods will be developed and finalized prior to the interim analysis.

9.2.1 Analysis of Subject Disposition, History, and Baseline Characteristics

Subject disposition, including analysis population allocation, subjects enrolled, completed each period, discontinued, and primary reason for discontinuation, will be summarized using frequency and percentage.

Protocol deviations will be summarized using frequency and percentage.

Medical history data and prior and concomitant medications will be summarized using frequency and percentage.

Subjects' age, height, weight, and baseline disease characteristics will be summarized using descriptive statistics. Gender, race, and other categorical variables will be provided using frequency and percentage.

9.2.2 Safety Analyses

All safety analyses will be performed on the Safety Population. The safety data will be presented in individual listings and summary tables.

Adverse Events

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.

TEAEs, AEs leading to study treatment discontinuation, AEs leading to dose interruption, AEs related to study medication, SAEs, and AEs leading to death will be summarized by system organ class, preferred term, and study period. A summary of AEs that are CTCAE Grade 3 or higher, as well as the most frequent preferred terms, will be provided.

If a subject experience the same preferred term multiple times within a period, then the event will be counted only once within the period and by greatest severity.

Descriptive statistics will be used to summarize the safety data both by treatment group and overall.

Clinical Laboratory Values

All laboratory test results will be summarized by period together with the change from baseline. The frequency distribution for low/normal/high or normal/abnormal will be summarized as well. The denominators for calculating the percentages will be based on the number of subjects with non-missing values in the Safety Population.

Vital Signs

Vital sign results will be summarized by period, together with the change from baseline.

Physical Examination

Summaries of physical examinations will present frequency distribution of abnormal findings by body system and 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.

Electrocardiogram (ECG)

ECG findings will be classified as normal vs abnormal. The number and percentage of each category will be summarized using frequency table for each period. The denominators for calculating the percentages will be based on the number of subjects with non-missing values in each period.

C-SSRS

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

9.2.3 Tolerability Analyses

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.

9.2.4 Efficacy Analyses

9.2.4.1 Primary Efficacy Endpoint Analyses

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 Efficacy Evaluable 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 (active or placebo) as a factor, and a subject's baseline weekly CSBM rate as a covariate. The comparison of active versus placebo treatment group will be tested at the two-sided 0.05 significance level. Summary statistics will also be presented.

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 primary efficacy analysis will also be performed on the mITT and Fixed Dose Populations.

As an additional supplemental analysis, the change from baseline in weekly CSBM rate using the subject's last 2 weeks of the Treatment Period will be analyzed using the ANCOVA model described for the primary endpoint using the mITT population.

9.2.4.2 Secondary Efficacy Endpoint Analyses

Secondary efficacy analyses will be based on the Efficacy Evaluable Population. The comparison of active versus placebo treatment will be tested at the two-sided 0.05 significance level.

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

All continuous secondary efficacy endpoints will be analyzed in the same manner as the primary efficacy endpoint.

The following are the set of dichotomous secondary efficacy endpoints:

- Pro-kinetic bowel response during the Fixed Dose period
- SBM bowel response to ENT-01 during the Fixed Dose period

For the dichotomous secondary efficacy endpoints, the comparison of the ENT-01 versus placebo treatment groups will be performed by means of Fisher's exact test.

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 secondary efficacy endpoints will be summarized using Kaplan-Meier methods, comparisons between treatment groups will be made via log-rank tests.
- For the frequency of dose adjustments in the Dose Adjustment period secondary endpoint, the frequency of the number of dose adjustments a subject has from the initial starting dose to the subject's fixed dose will be summarized by treatment group and baseline CSBM strata.
- For the PAC-SYM and PAC-QOL subjective assessments secondary efficacy endpoints, the overall scores from the Fixed Dose period will be analyzed via an ANCOVA model with treatment as a factor and the subject's corresponding baseline score as a covariate. Additional summaries of the individual items will be performed.

9.2.4.3 Exploratory Efficacy Endpoint Analyses:

The analysis methods for the exploratory efficacy endpoints will be provided in the SAP.

9.2.5 Sample Size/Power Considerations

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. In that study, of the 72 randomized subjects, 64 (89%) were evaluable for the primary efficacy endpoint analysis. Randomization was 3:1 to active treatment or placebo; 64

subjects were randomized to active treatment and 18 subjects were randomized to placebo. The

study assumed an increase from baseline in weekly CSBM rate in the Fixed Dose period of 2.4 and 0.5 for the active drug and placebo-treated subjects, respectively, and an SD of 2.0.

amended.

In this protocol amendment, enrolling an additional 80 subjects in a 1:1 randomization will yield a conditional power of approximately 93% and an additional 60 subjects will yield a conditional power of 88%. This is based on the additional subjects having a least squares mean change from baseline for the Fixed Dose Period of 3.55 and 1.28 for ENT-01 and placebo, respectively, and a common standard deviation of 3.88. The power calculations incorporated a 10% drop-out rate. These parameter estimates and conditional power calculations are based on the results of the initial cohort of subjects in the Phase 2b study.

9.2.6 Data Cut Analyses

An unblinded interim analysis was performed after the initial cohort of the study. An unblinded interim analysis may be performed after the 40th subject (in the 1:1 randomized cohort) completes the Fixed Dose period (i.e., Visit 4).

10 ESTIMATED DURATION OF THE STUDY

This study has an estimated maximum duration of up to 10 weeks for each subject. Maximum number of active dosing days for each subject will be no more than 25 days. The study duration from first subject in, to last subject out is expected to be 18 months.

11 ADMINISTRATIVE PROCEDURES AND ETHICAL CONSIDERATIONS

11.0 Subject Information and Informed Consent

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

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

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

11.1 Study Monitoring

During the study, a monitor from Enterin, Inc. or representative will have regular contacts with the investigational site, for the following:

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

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

11.2 Audits and Inspections

Authorized representatives of Enterin, Inc., a regulatory authority, an Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the site to perform audits or inspections, including source data verification. The purpose of an Enterin, Inc. audit or

inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP) guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements. The investigator should contact Enterin, Inc. immediately if contacted by a regulatory agency about an inspection.

11.3 Ethics Committee Review

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

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

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

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

11.4 Standards

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/GCP, applicable regulatory requirements.

11.5 Confidentiality

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

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

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

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

11.6 Protocol Adherence

The site will maintain records of study treatment delivered to the study site; the inventory at the site; the distribution to and use by each subject; and the return of materials to Enterin, Inc. for storage or disposal. These records should include dates, quantities, batch/serial numbers, expiration dates, in-clinic temperature log, and unique code numbers assigned to the product and study subjects.

At each visit after initiation of treatment, site staff will record compliance of subjects with their assigned regimen. Subjects will be instructed to return their diaries and unused/partially used/empty bottles for inspection at each study visit. Subjects are to be reminded of the importance of compliance with their assigned regimen, with an emphasis on taking their study drug on schedule and maintaining the prescribed interval between doses.

Investigator will maintain records that document adequately that the subjects were provided with the correct study drug and will reconcile the products received from the drug dispensing

center. Investigational product will not be returned to Enterin, Inc. until accountability has been fully monitored.

Medication containers must be returned at each visit, as compliance will be assessed by tablet counts. Noncompliance is defined as taking less than 80% or more than 120% of study drug during any out-patient evaluation period (visit to visit). Discontinuation for noncompliance is at the Investigator's discretion and is to be noted on the CRF.

11.7 Amendments to the Protocol

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

11.8 Protocol Deviations

The protocol must be read thoroughly, and the instructions followed exactly. The sponsor and/or designee will not grant waivers for protocol deviations. Any deviation to the protocol must be reported as soon as possible to the sponsor and to the IRB per IRB reporting requirements. These reporting guidelines for protocol deviations must be adhered to by the Investigator.

11.9 Study Termination

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

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Failure to enroll subjects at an acceptable rate.
- Insufficient adherence to protocol requirements.
- Insufficient complete and/or evaluable data.
- Plans to modify, suspend, or discontinue the development of ENT-01.

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

12 DATA HANDLING AND RECORD KEEPING

12.0 Inspection of Records

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

12.1 Data Management

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

The data cut-off for the study will be 60 days after entry of the last subject. If subjects remain on study thereafter, a revised final study report will be issued after the last subject has completed the final study evaluation.

12.2 Data Capture and Management

The sponsor will provide the study sites with an electronic case report system.

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

The Investigator, or designated representative, should complete the eCRF as soon as possible after information is collected, preferably on the same day that a subject is seen for an

examination, treatment, or any other study procedure. Any outstanding entries must be entered immediately after the final examination. An explanation should be given for all missing data.

12.3 Liability and Insurance

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

12.4 Retention of Records

Study records and source documents must be preserved for the longer of (i) two (2) years following completion of the study; or (ii) two (2) years following the termination or withdrawal of the Investigational New Drug application under which this study was conducted; or (iii) the period required by local, state, and federal laws, regulations and FDA Guidance.

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

12.5 Data Quality Assurance

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

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

If sites receive a request for an inspection or written or oral inquiries regarding any aspect of

institution's or Investigator's activities related to this study from a regulatory authority, the Investigator must immediately notify the sponsor and the appropriate CRO of the request. Following this inspection and/or audit, the Investigator must notify the sponsor of any violation or deficiency noted by the regulatory authority.

13 USE OF INFORMATION

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

14 REFERENCES

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APPENDIX 1 LABORATORY TESTS

Hematology	**Serum Chemistry**	**Urinalysis**
White Blood Cell (WBC) Count	Albumin	Appearance
Red Blood Cell (RBC) Count	Alanine Aminotransferase (ALT)	pH
Hemoglobin (Hb)	Alkaline Phosphatase (ALP)	Protein
Hematocrit (Hct)	Aspartate Aminotransferase (AST)	Glucose
Mean Corpuscular Volume (MCV)	Blood Urea Nitrogen (BUN)	Ketone Bodies
Red Blood Cell Distribution Width	Ratio	Indicators of Blood and WBCs
(RDW)	Calcium	Specific Gravity
Platelet Count	Carbon Dioxide	Urobilinogen
Differential - absolute and percent	Chloride	
of:	Creatinine	
Neutrophils	Glucose	
Lymphocytes	Potassium	
Monocytes	Sodium	
Eosinophils	Total Bilirubin	
Basophils	Total Protein	
PT		
PTT		
INR		
	Urea Nitrogen	
	Lactate Dehydrogenase (LDH)	
	Creatine Kinase, Total	

Pregnancy tests
A serum pregnancy test will be performed on all female subjects of child-bearing potential at the screening visit. Urine Pregnancy Test (on site): Urine human chorionic gonadotropin (HCG) (pre-menopausal females only)
Follicle Stimulating Hormone (FSH) for post-menopausal women under age 60.

APPENDIX 2 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 -21	Day 1 (+/-8 days)	Day 11 (+/- 3 day)	Day 26 (- 2 days)	Day 39 (+/- 8 days)	Day 69 (+/- 8 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 -21	**Day 1 (+/-8 days)**	**Day 11 (+/- 3 day)**	**Day 26 (- 2 days)**	**Day 39 (+/- 8 days)**	**Day 69 (+/- 8 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			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 approximately 5 minutes. Sitting or standing measurements will be taken within approximately 5 minutes of sitting or standing.

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".

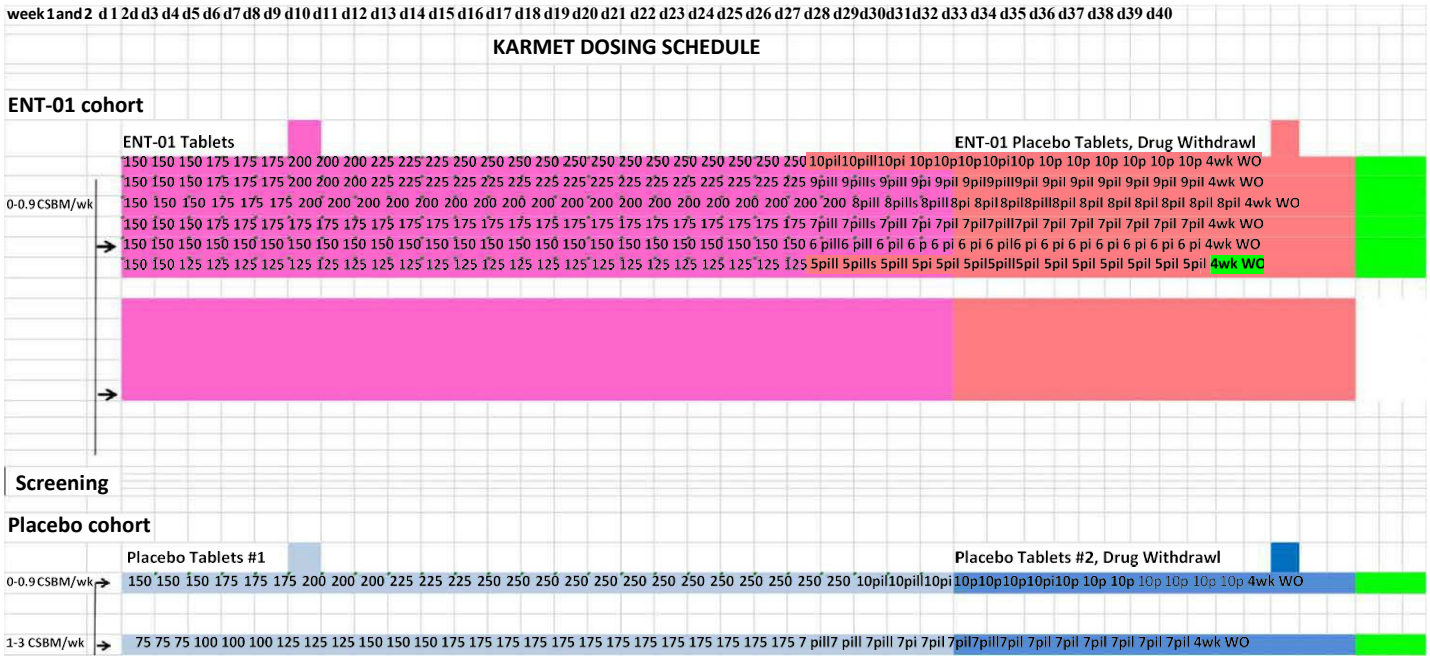
i. Those subjects who have a SAPS-PD score >0 on Visits 1 and 2 will have the SAPS-PD and hallucination/delusion questionnaire at all subsequent visits.

- 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 subjects discontinues from the study prior to the EoT visit, a stool sample should be collected, as applicable, at the Early Termination visit.

AE=adverse event; BDI-II= Beck Depression Inventory II; BM=bowel movement; BP=blood pressure; C-SSRS= Columbia-Suicide Severity Rating Scale; ECG=electrocardiogram; EoT=end of treatment; FSH=follicle-stimulating hormone; HR=heart rate; L-Dopa=levodopa; MDS-UPDRS=Movement Disorder Society's Unified Parkinson's Disease Rating Scale; MMSE=Mini-mental State Examination; PAC-QoL=Patient Assessment of Constipation - Quality of Life;
PAC-SYM=Patient Assessment of Constipation Symptoms; PK=pharmacokinetic(s); RR=respiratory rate; SAPS-PD=Scale for Assessment of Positive Symptoms – Parkinson's Disease; UPT=urine pregnancy test.

APPENDIX 3 DOSING SCHEDULE



APPENDIX 4 ROME-IV CRITERIA FOR CONSTIPATION

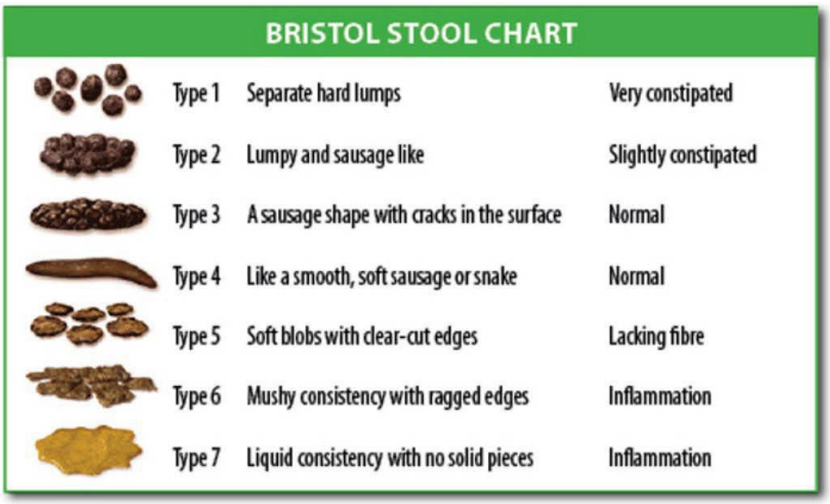
Must include two or more of the following:
Straining during at least 25% of defecations
Lumpy or hard stools in at least 25% of defecations
Sensation of incomplete evacuation for at least 25% of defecations
Sensation of anorectal obstruction/block age for at least 25% of defecations
Manual maneuvers to facilitate at least 25%of defecations (e.g., digital evacuation, support of the pelvic floor)
Fewer than 3 defecations per week
Loose stools are rarely present without the use of laxatives

APPENDIX 5 EASE OF PASSAGE

1.Manual disimpaction
2.Enema needed
3.Straining needed
4.Normal
5.Urgent without pain
6.Urgent with pain
7.Incontinent

APPENDIX 6 BRISTOL STOOL CHART

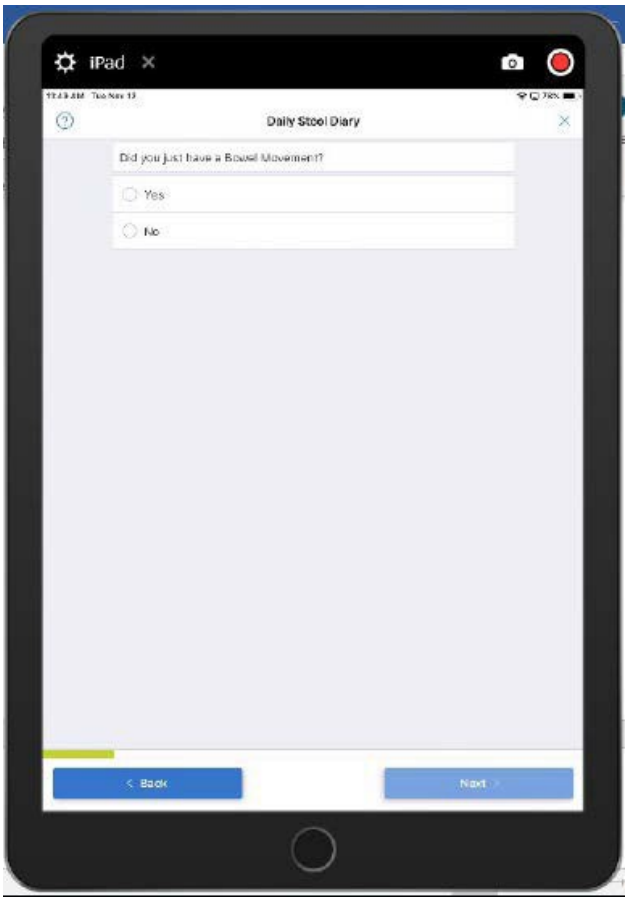
The assessment of stool consistency is a validated surrogate of intestinal motility (Lewis and Heaton, 1997). We have chosen the Bristol Stool Chart as a subject-friendly means of categorizing stool characteristics.

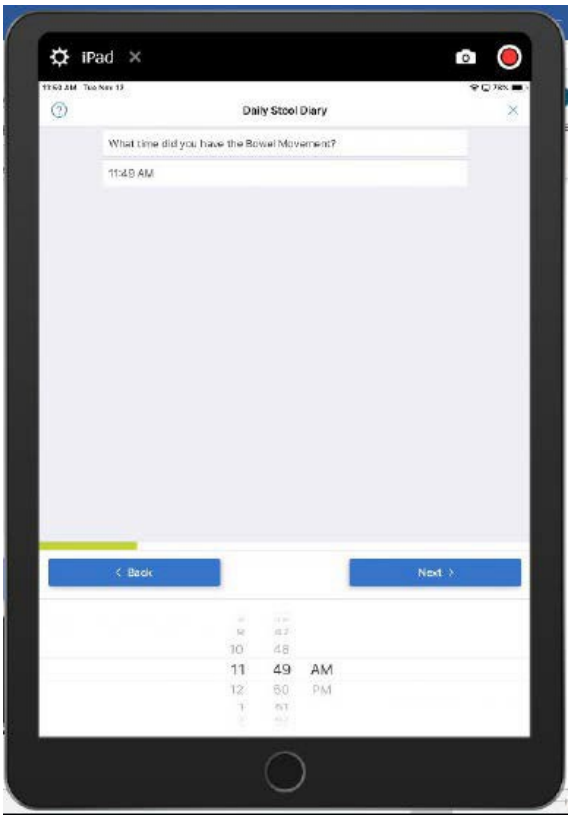


APPENDIX 7 SUBJECT DIARIES

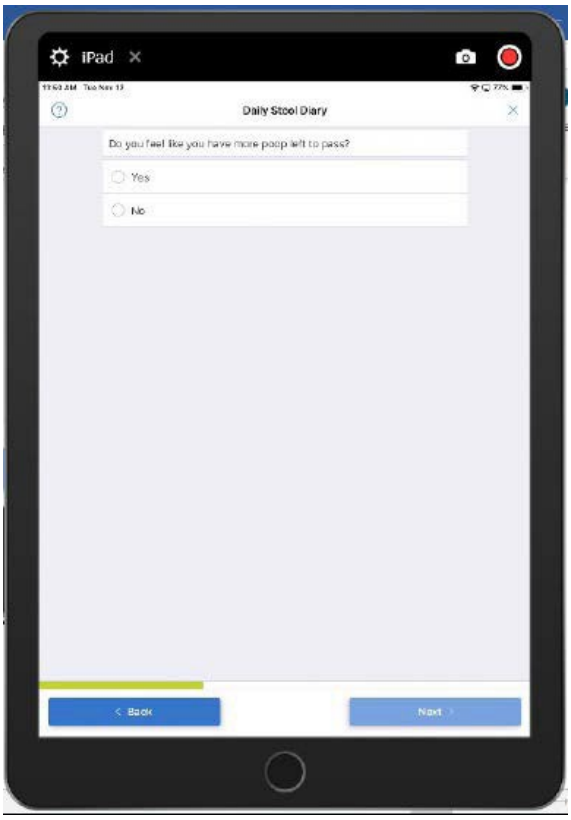
Participants will complete a stool diary on an IPAD tablet on a daily basis throughout the study. The diaries will include information about whether the stool was a CSBM as well information about consistency, ease, laxative and rescue medication use.

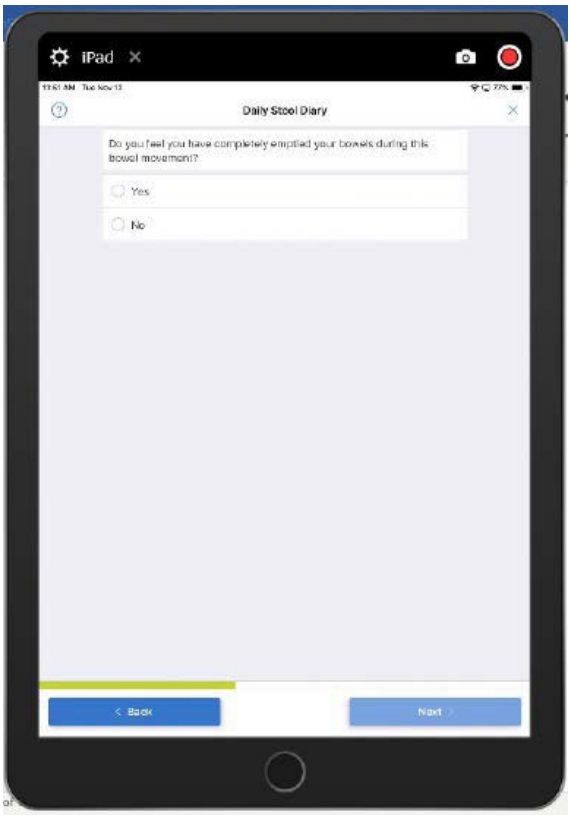

iPad
Daily Stool Diary
Please take your pills as soon as you wake up with 8 ounces of water. Do not eat any food for 60 minutes after taking the pills. You are free to take tea or coffee with or without milk and/or sugar. Please remain in a standing or sitting position for 60 minutes after taking the medication. Do not go back to bed. Do not lie down.
Next >

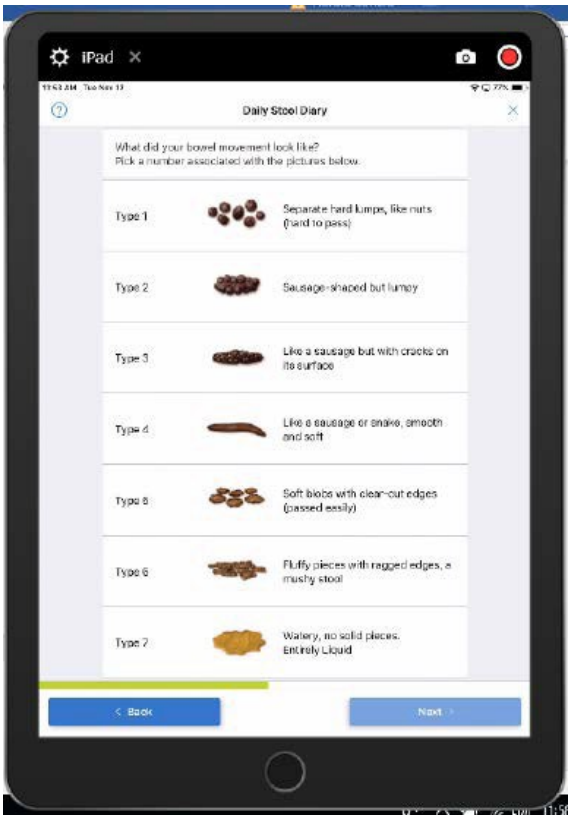

iPad
Daily Stool Diary
Did you just have a Bowel Movement?
Yes
No
< Back
Next >

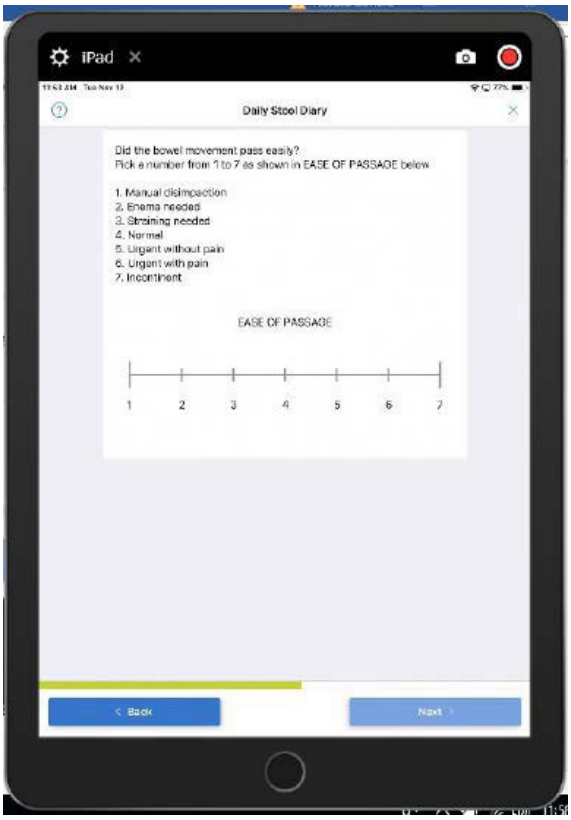

iPad
Daily Stool Diary
What time did you have the Bowel Movement?
11:49 AM
Back
Next
10 48
11 49 AM
12 50 PM


iPad
Daily Stool Diary
Are you done for the day?
Yes
No
< Back
Next >


iPad
Daily Stool Diary
Do you feel like you have more poop left to pass?
Yes
No
< Back
Next >


iPad
Daily Stool Diary
Do you feel you have completely emptied your bowels during this bowel movement?
Yes
No
< Back
Next >

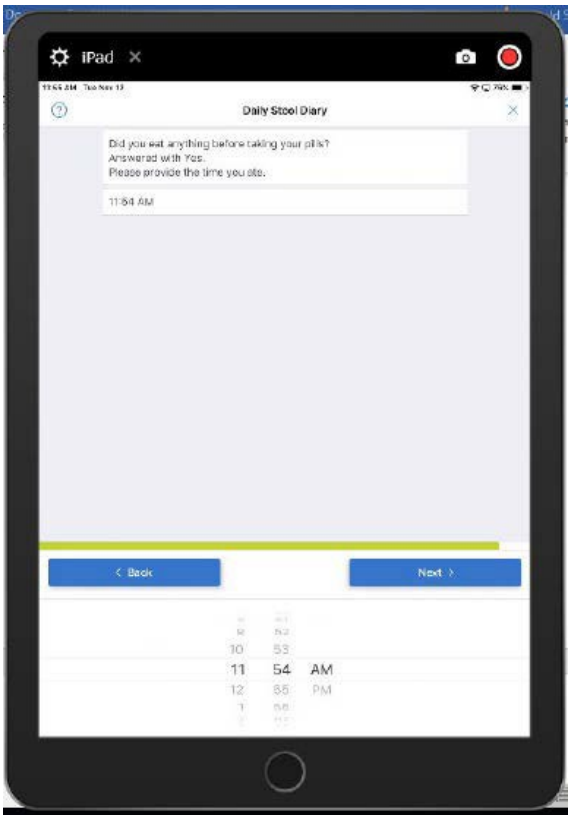

iPad
Daily Stool Diary
What did your bowel movement look like?
Pick a number associated with the pictures below.
Type 1
Separate hard lumps, like nuts (hard to pass)
Type 2
Sausage-shaped but lumpy
Type 3
Like a sausage but with cracks on its surface
Type 4
Like a sausage or snake, smooth and soft
Type 5
Soft blobs with clear-cut edges (passed easily)
Type 6
Fluffy pieces with ragged edges, a mushy stool
Type 7
Watery, no solid pieces. Entirely Liquid
< Back
Next >

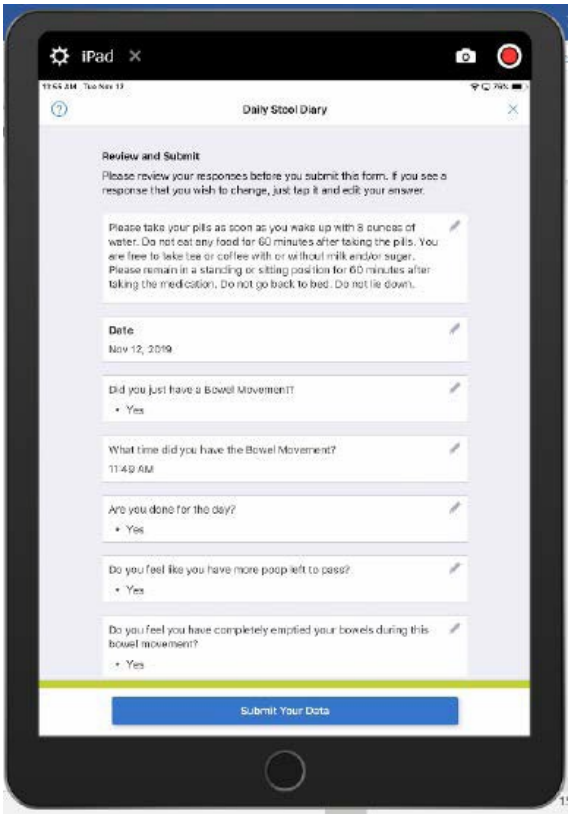

iPad
Daily Stool Diary
Did the bowel movement pass easily?
Pick a number from 1 to 7 as shown in EASE OF PASSAGE below
1. Manual disimpaction
2. Enema needed
3. Straining needed
4. Normal
5. Urgent without pain
6. Urgent with pain
7. Incontinent
EASE OF PASSAGE
1 2 3 4 5 6 7
< Back
Next >


iPad
Daily Stool Diary
Did you take your Study Medication this morning?
Yes
No
< Back
Next >

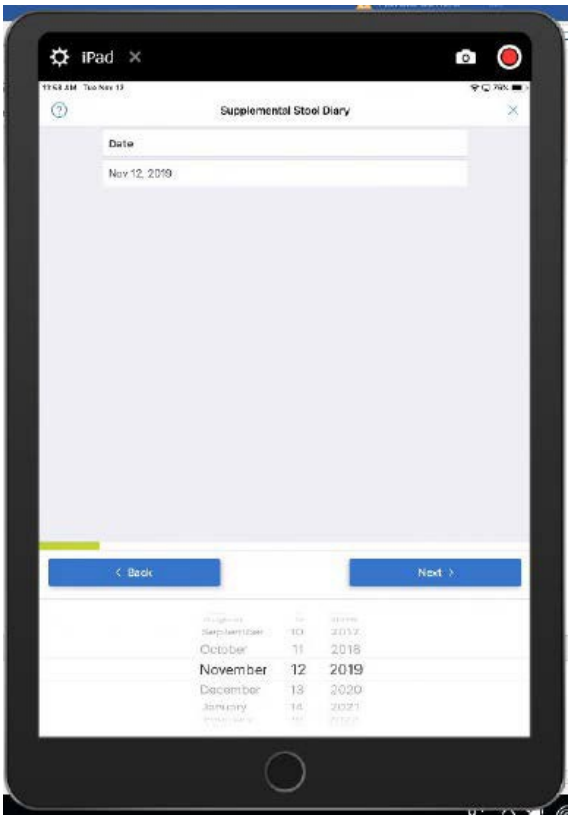

iPad
Daily Stool Diary
What time did you take your dose?
11:53 AM
Back
Next
10
11
12
52
53
54
AM
PM

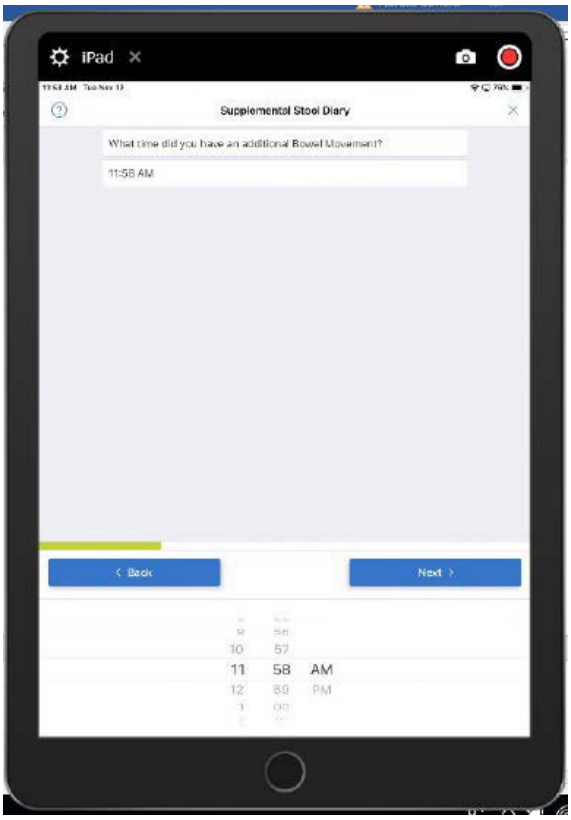

iPad
Daily Stool Diary
Number of Tablets Taken
0
< Back
Next >
1
2
3
4
5
6
7
8
9
0


iPad
Daily Stool Diary
Did you eat anything before taking your pills?
Answered with Yes.
Please provide the time you ate.
11:54 AM
Back
Next
10
11
12
53
54
55
AM
PM

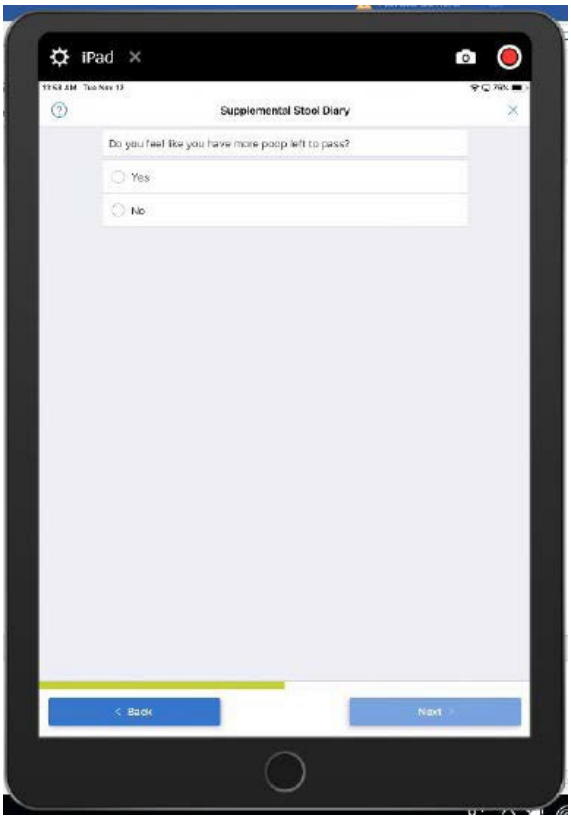

iPad
Daily Stool Diary
Review and Submit
Please review your responses before you submit this form. If you see a response that you wish to change, just tap it and edit your answer.
Please take your pills as soon as you wake up with 8 ounces of water. Do not eat any food for 60 minutes after taking the pills. You are free to take tea or coffee with or without milk and/or sugar. Please remain in a standing or sitting position for 60 minutes after taking the medication. Do not go back to bed. Do not lie down.
Date
Nov 12, 2019
Did you just have a Bowel Movement?
• Yes
What time did you have the Bowel Movement?
11:49 AM
Are you done for the day?
• Yes
Do you feel like you have more poop left to pass?
• Yes
Do you feel you have completely emptied your bowels during this bowel movement?
• Yes
Submit Your Data

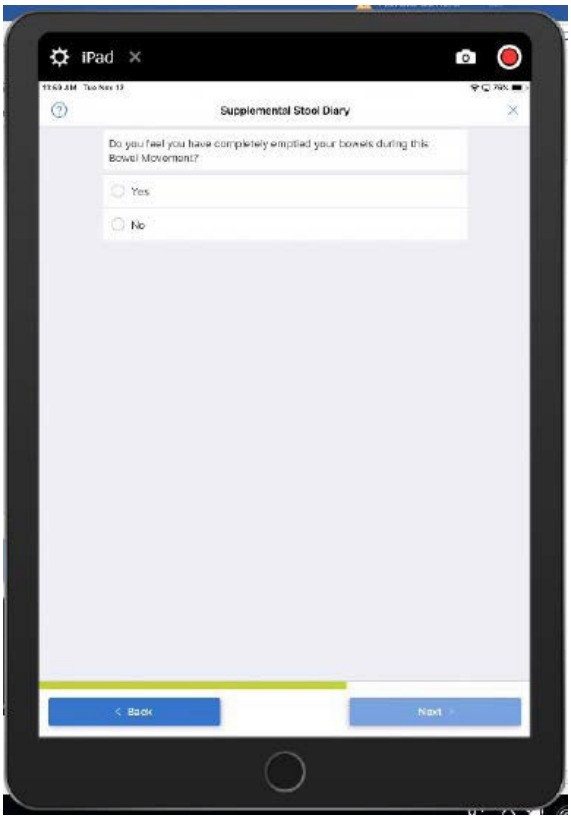

iPad
Supplemental Stool Diary
Please submit this Supplemental Stool Diary form if you have already filled out the Daily Stool Diary today, and for each additional BM today.
If you have not filled out the Daily Stool Diary today, please exit this Supplemental Stool Diary form and fill out the Daily Stool Diary.
Next >

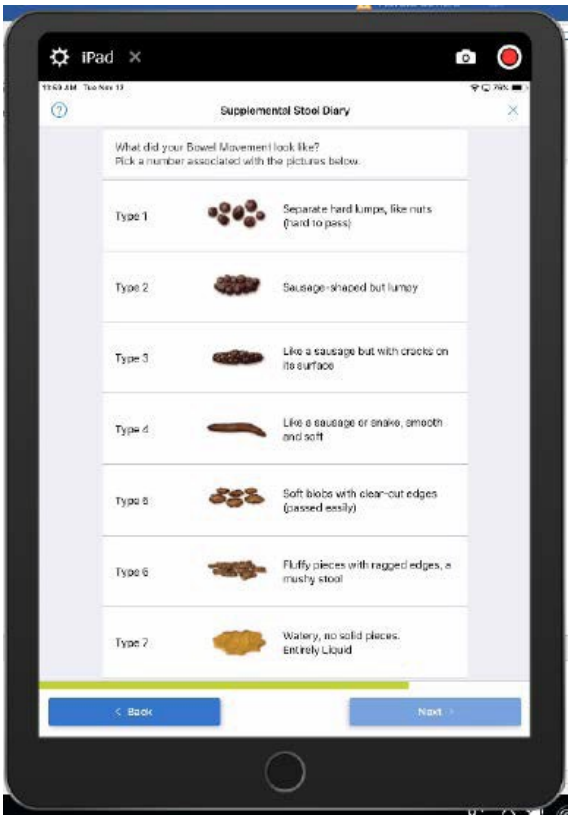

iPad
Supplemental Stool Diary
Date
Nov 12, 2019
Back
Next
October 11 2018
November 12 2019
December 13 2020

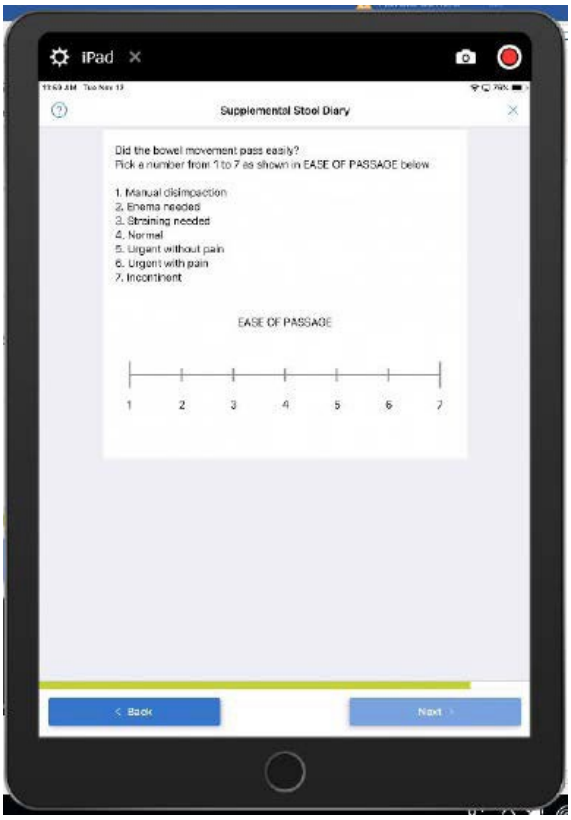

iPad
Supplemental Stool Diary
What time did you have an additional Bowel Movement?
11:58 AM
Back
Next
10
11
12
57
58
59
AM
PM

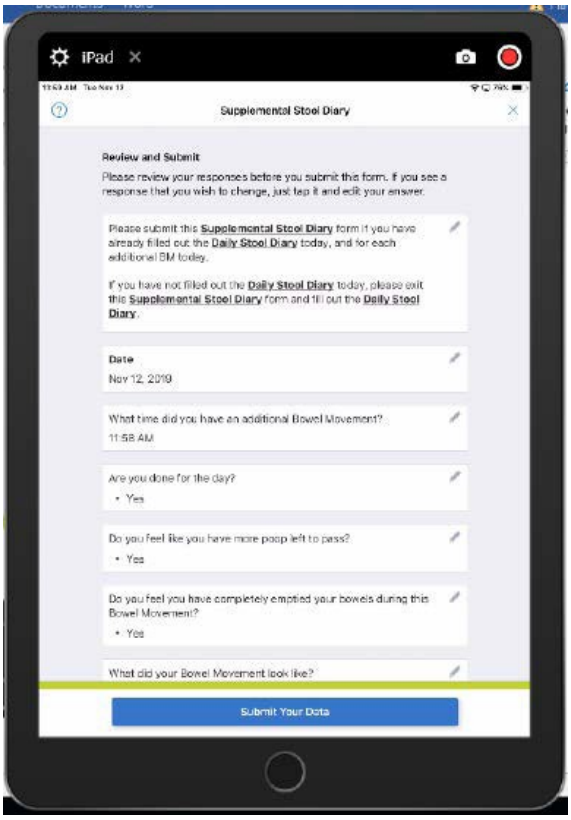

iPad
Supplemental Stool Diary
Are you done for the day?
Yes
No
< Back
Next >

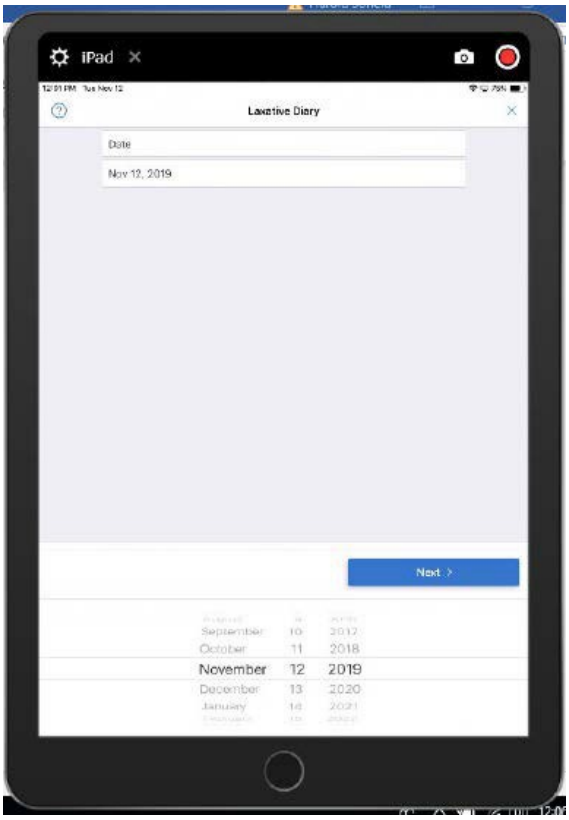

iPad
Supplemental Stool Diary
Do you feel like you have more poop left to pass?
Yes
No
< Back
Next >

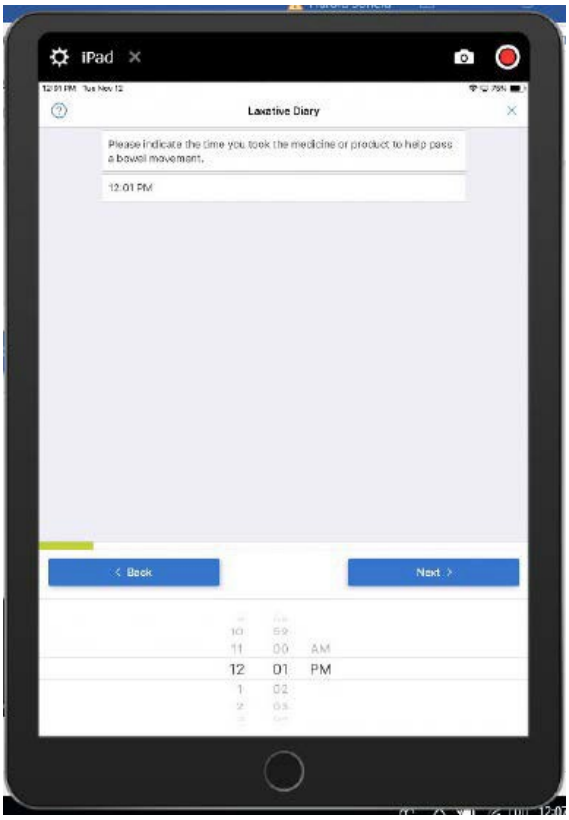

iPad
Supplemental Stool Diary
Do you feel you have completely emptied your bowels during this Bowel Movement?
Yes
No
< Back
Next >


iPad
Supplemental Stool Diary
What did your Bowel Movement look like?
Pick a number associated with the pictures below.
Type 1
Separate hard lumps, like nuts (hard to pass)
Type 2
Sausage-shaped but lumpy
Type 3
Like a sausage but with cracks on its surface
Type 4
Like a sausage or snake, smooth and soft
Type 5
Soft blobs with clear-cut edges (passed easily)
Type 6
Fluffy pieces with ragged edges, a mushy stool
Type 7
Watery, no solid pieces. Entirely Liquid
< Back
Next >


iPad
Supplemental Stool Diary
Did the bowel movement pass easily?
Pick a number from 1 to 7 as shown in EASE OF PASSAGE below
1. Manual disimpaction
2. Enema needed
3. Straining needed
4. Normal
5. Urgent without pain
6. Urgent with pain
7. Incontinent
EASE OF PASSAGE
1 2 3 4 5 6 7
< Back
Next >

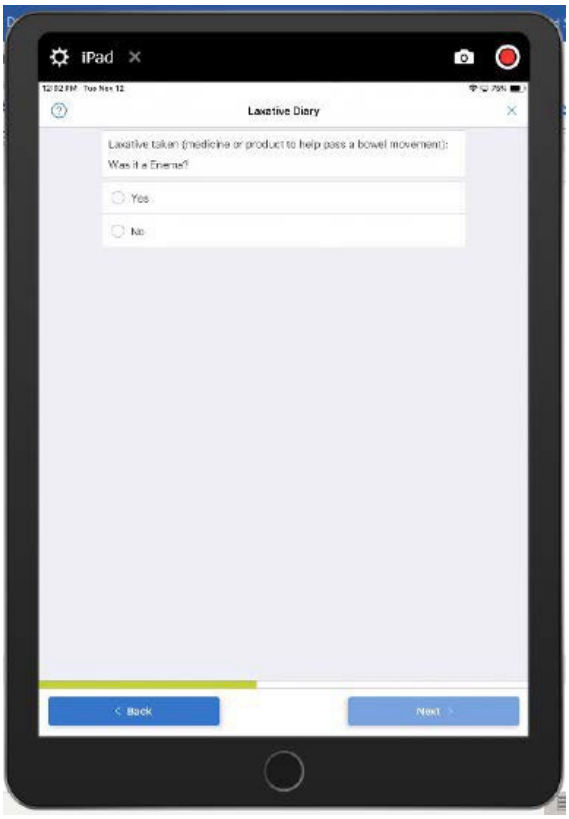

iPad
Supplemental Stool Diary
Review and Submit
Please review your responses before you submit this form. If you see a response that you wish to change, just tap it and edit your answer.
Please submit this Supplemental Stool Diary form if you have already filled out the Daily Stool Diary today, and for each additional BM today.
If you have not filled out the Daily Stool Diary today, please exit this Supplemental Stool Diary form and fill out the Daily Stool Diary.
Date
Nov 12, 2019
What time did you have an additional Bowel Movement?
11:58 AM
Are you done for the day?
• Yes
Do you feel like you have more poop left to pass?
• Yes
Do you feel you have completely emptied your bowels during this Bowel Movement?
• Yes
What did your Bowel Movement look like?
Submit Your Data


iPad
Laxative Diary
Date
Nov 12, 2019
Next >
September 10 2017
October 11 2018
November 12 2019
December 13 2020
January 14 2021

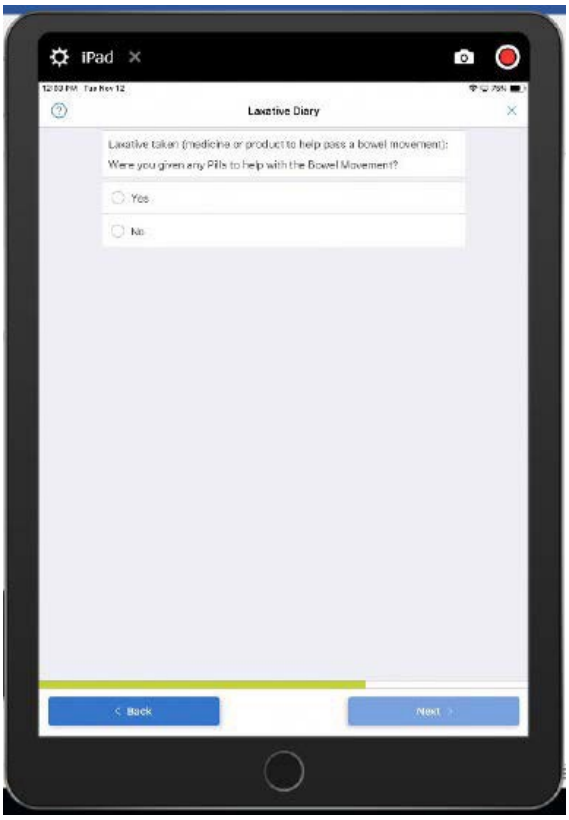

iPad
Laxative Diary
Please indicate the time you took the medicine or product to help pass a bowel movement.
12:01 PM
< Back
Next >
10 59
11 00 AM
12 01 PM
1 02


iPad
Laxative Diary
Laxative taken (medicine or product to help pass a bowel movement):
Was it a Suppository?
Yes
No
< Back
Next >

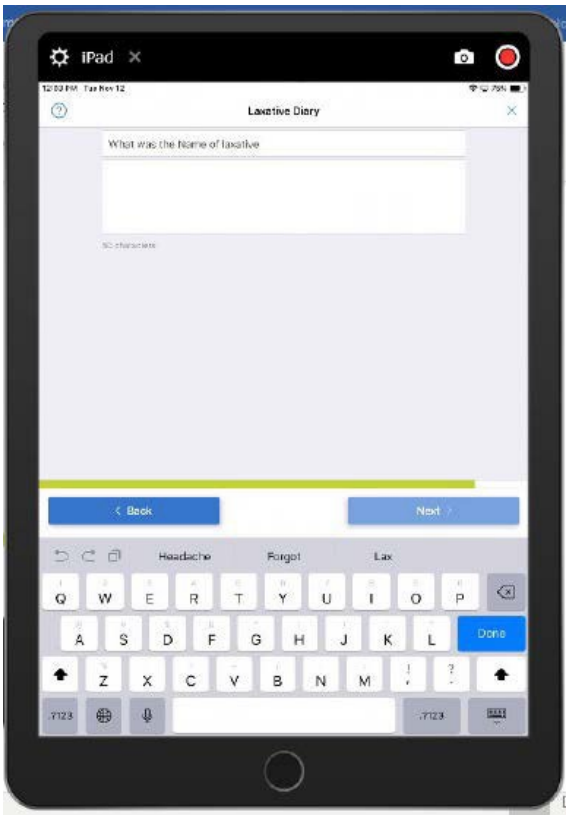

iPad
Laxative Diary
How many did you use?
poo
Back
Next
1
2
3
4
5
6
7
8
9
0

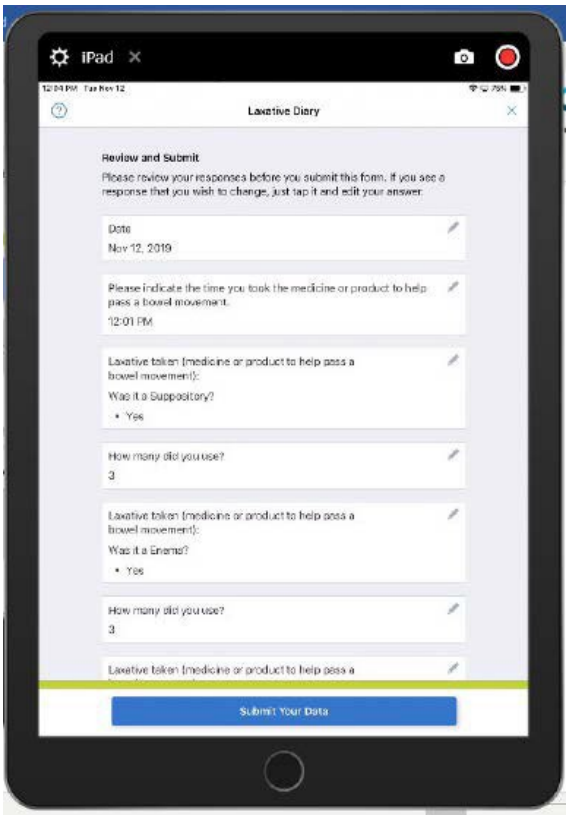

iPad
Laxative Diary
Laxative taken (medicine or product to help pass a bowel movement):
Was it a Enema?
Yes
No
Back
Next


iPad
Laxative Diary
How many did you use?
poo
Back
Next
1
2
3
4
5
6
7
8
9
0


iPad
Laxative Diary
Laxative taken (medicine or product to help pass a bowel movement):
Were you given any Pills to help with the Bowel Movement?
Yes
No
Back
Next


iPad
Laxative Diary
Laxative taken (medicine or product to help pass a bowel movement):
Were you given any Liquid laxative to take by mouth?
Yes
No
Back
Next


iPad
Laxative Diary
What was the Name of laxative
Back
Next
Headache
Forgot
Lax
Done


iPad
Laxative Diary
Review and Submit
Please review your responses before you submit this form. If you see a response that you wish to change, just tap it and edit your answer.
Date
Nov 12, 2019
Please indicate the time you took the medicine or product to help pass a bowel movement.
12:01 PM
Laxative taken (medicine or product to help pass a bowel movement):
Was it a Suppository?
• Yes
How many did you use?
3
Laxative taken (medicine or product to help pass a bowel movement):
Was it a Enema?
• Yes
How many did you use?
3
Laxative taken (medicine or product to help pass a
Submit Your Data

APPENDIX 8 PD HALLUCINATION QUESTIONNAIRE

Many patients with Parkinson's disease suffer from what we call hallucinations or apparitions. We would like to ask you some questions regarding hallucinations.

a. Do you sometimes feel someone is present who isn't actually there?

b. Do you sometimes think someone went by who didn't? If so, any one in particular?

c. Do you see things not seen by others? If so, how many months or years have you been seeing things? what do you see? People? Animals? Relatives? Children? When do you see them? Daytime? Evening? At night? Are they bothersome? Do they upset you? How often does this occur, many times a day? every day? Twice a week? Once a month? Can you provide a narrative description of them below?

d. Do you mistake objects for animals, like a white cloth for a white cat?

e. Do you feel like someone is going to harm you or is out to get you?

f. Do you feel things such as insects crawling up your legs on your hands or strange sensations such as scratching or burning or tingling on your hands?

g. Do you hear voices that aren't actually there? Do the voices talk to you? To each other? Talk about you?

h. Do you smell any abnormal smells not smelled by others? Are they unpleasant smells?

Please describe your experience in detail (i.e. what do you hear, see, smell or feel? Make sure you note how many times a day or night or week any of these episodes occur)

i. Have you been diagnosed with any eye disease? How good is your eyesight? If it's poor, how bad is it? How long have you had poor eyesight? How fast has it been getting worse?

ii. Do you experience hallucinations while "on" or "off"?

iii. Has your dopamine dose been changed recently?

iv.

v.

Interviewer's Signature:_____ Date:_____/_____/_____

Title:_____

PI Review of Interview

Once completed by or reviewed by the PI, please enter any observations or notes of significance regarding this patient.

PI Signature:_____ Date:_____/_____/_____

DD MMM YYYY