A Multicenter, Non-Randomized, Open-Label Study to Evaluate the Safety and Efficacy of Orally Administered ENT-01 in Improving Constipation and Neurologic Symptoms in Patients with Parkinson's disease and Constipation over a 14-week Period.

Clinical Trial Protocol

Name of product	ENT-01
Phase	Phase 2b Follow-on Safety "Roll-over" study
Trial Protocol Number	ENT-01-2b-20-02
IND Number	130770
Sponsor	Enterin, Inc. 2005 Market St. Suite 3125 Philadelphia, PA 19103
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Clinical Trial Protocol Version / Date	Version 3.0 / June 30th, 2020
Protocol Amendment Number / Date	NA

CONFIDENTIALITY STATEMENT

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

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CLINICAL TRIAL PROTOCOL SIGNATURE PAGE

Authored by:

Signature _____

Date of Signature _____

(DD/MMM/YY)

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Investigator Agreement

I have read this protocol "A Multicenter, Non-Randomized, Open-Label Study to Evaluate the Safety and Efficacy of Orally Administered ENT-01 in Improving Constipation and Neurologic Symptoms in Patients with Parkinson's disease and Constipation over a 14-week Period," and any auxiliary materials related to Study ENT-01-2b-20-02, and agree to the following:

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

_____

Investigator's Signature Date

STUDY SYNOPSIS

Study Title	A Multicenter, Non-Randomized, Open-Label Study to Evaluate the Safety and Efficacy of Orally Administered ENT-01 in Improving Constipation and Neurological Symptoms in Patients with Parkinson's disease and Constipation over a 14-week Period.
Protocol Number	ENT-01-2b-20-02
Study Phase	Phase 2b Follow-on study.
Methodology	This study will be conducted as an open-label safety follow-on to a multi-center, double-blind, randomized study. Dosing duration will be approximately 14 weeks. All subjects who participated in the randomized study will be offered participation in this unblinded, single-arm, safety study. ENT-01 will be administered daily in escalating doses followed by a fixed-dose for 12 weeks. The study will be conducted on an out-patient or in-patient basis with visits performed at the screening visit, at 6 and 12 weeks, and at the end of the 6th week of the wash-out period (end of study).
Study Duration	Each subject will participate for approximately 20 weeks: the dose escalation period lasting up to 20 days, the fixed dose period lasting 12 weeks, and the wash-out period lasting 6 weeks.
Study Centers	This is a multi-center trial with approximately 20 sites.
Objectives	The purpose of this study is to evaluate the long-term safety and efficacy of orally administered ENT-01 in subjects with Parkinson's disease and constipation. **Primary Safety Objective:** To determine the safety and tolerability of orally administered ENT-01 for up to 14 weeks in subjects with Parkinson's disease-related constipation. **Primary Efficacy Objectives:** To determine the efficacy of repeated oral doses of ENT-01 in improving constipation in subjects with Parkinson's disease. **Secondary Efficacy Objectives:** To determine the effect of repeated oral doses of ENT-01 on motor and non-motor symptoms such as cognition and psychosis in subjects with Parkinson's disease.

Number of Subjects	Approximately 50 subjects will be entered into the study
Study Description	Open-Label, Multicenter study of daily oral doses of ENT-01 for up to 14 weeks
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. All subjects who participated in the randomized study ENT-01-030 (KARMET Stage 2 Extension subjects) and who completed the dosing period. 2. Subjects aged 30-90 years at the time of screening for the ENT-01-030 study, both genders. 3. Subjects must provide informed consent and be willing and able to comply with study procedures. 4. Subjects must be able to read, understand, and accurately record data into the diary to guarantee full participation in the study. 5. Female subjects must have negative serum or urine pregnancy tests and must not be lactating. For females able to bear children, a hormonal (i.e., oral, implantable, or injectable) and 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. 6. 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. Unable to withdraw proton pump inhibitors. 3. 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. 4. Diagnosis of secondary constipation beyond that of Parkinson's disease. 5. 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). 6. Neurological disorder other than Parkinson's disease that in the opinion of the investigator might interfere with the conduct of the study. 7. On treatment with intra-jejunal dopamine or carbidopa/levodopa (i.e. Duopa). 8. Subjects starting a new Parkinson's disease medication or modifying an existing medication within 2 weeks prior to enrollment. 9. Unable to maintain a stable diet regimen. 10. Subjects with a cognitive impairment that preclude them from understanding the informed consent. 11. Subjects placed under legal guardianship. 12. History of excessive alcohol use or substance abuse. 13. Any clinically significant abnormalities on screening laboratories or physical examination requiring further evaluation or treatment.

	14. Females who are pregnant or breastfeeding 15. Subject or caregiver unable to administer daily oral dosing of study drug. 16. Participation in a non-Enterin 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 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. The tablets will be 25mg each. Subjects will begin dosing at the same dose level they were stratified to in the ENT-01-030 randomized study (*i.e.*, starting at either 3 tablets or 6 tablets,) and escalate up to a pro-kinetic dose or a maximum dose of 250mg.
Study Endpoints	**Safety Endpoints:** Adverse events as evaluated with subject report, vital signs, chemical chemistry and electrocardiograms (EKG). **Tolerability Endpoints:** • Recurrent vomiting defined as 3-5 episodes of vomiting within 24 hours. • Recurrent diarrhea defined as 7 episodes of diarrhea within 24 hours for 2 consecutive days. • Dizziness defined as lightheadedness or fainting on rising from lying to sitting or standing and severe enough to require non-urgent medical intervention within 24 hours. **Primary Efficacy Endpoint:** • Change from baseline in weekly CSBM rate during the 12 weeks of treatment over baseline. **Secondary Efficacy Endpoints:** • Change from baseline in the MDS-UPDRS score at the end of the 12-week fixed dose period. • Change from baseline in cognition as assessed by the MMSE at the 12-week fixed dose period.

	• Change from baseline in psychosis as assessed by the SAPS-PD score at the 12-week fixed dose period.
Statistical Considerations	**Study Populations:** • Safety Population: All enrolled subjects who have received at least 1 dose of study drug. • Fixed Dose Population: The Fixed Dose Population will include all subjects who are in Safety Population who enter the Fixed Dose period of the study. • Efficacy Evaluable Population: All subjects in the ITT Population who complete the study with no major protocol violations. **Analysis Methods:** Descriptive summary statistics will be presented for the safety and tolerability endpoints overall. Continuous variables will be summarized using the number of observations, mean, standard deviation, median, minimum, and maximum. Categorical variables will be summarized using frequency counts and percentages. **Primary Efficacy Endpoint Analysis:** The primary efficacy endpoint is the change from KARMET Stage 2 baseline in the weekly CSBM rate during 12 Weeks of the Treatment Period. The primary efficacy endpoint analysis is a Mixed Model Repeated Measures (MMRM) analysis. **Sample Size Justification:** To assess tolerability and safety, a sample size of 50 patients yields 92% probability that an AE with an underlying rate of 5% will occur in at least one of these patients.
Initial Date	July 2020
Completion Date	December 2021

Table of Contents

Tables and Figures

ABBREVIATIONS

ADL	Activity of Daily Living
AE	Adverse Event
αS	Alpha-synuclein
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CNS	Central Nervous System
COMT	Catechol-O-methyltransferase
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organization
CSBM	Complete Spontaneous Bowel Movement
CTCAE	Common Terminology Criteria for Adverse Events
DEP	Dose Escalation Period
DLT	Dose Limiting Toxicity
DSMB	Data Safety and Monitoring Board
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EKG	Electrocardiogram
ENS	Enteric Nervous System
ET	Early Termination
FDA	Food and Drug Administration
FDP	Fixed Dose Period
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GI	Gastrointestinal
HDPE	High Density Polyethylene
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IND	Investigational New Drug
IP	Investigational Product
IPAN	Intrinsic Primary Afferent Neurons
IRB	Internal Review Board
ITT	Intention to Treat
MedRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed Model Repeated Measures

MMSE	Mini Mental State Examination
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NOAEL	No Observable Adverse Effect Level
PD	Parkinson's Disease
RBD	REM Behavior Disorder
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAPS-PD	Scale for the Assessment of Positive Symptoms for Parkinson's Disease Psychosis
SD	Standard Deviation
SNRI	Serotonin Norepinephrine Reuptake Inhibitor
SSRI	Selective Serotonin Reuptake Inhibitor
TEAE	Treatment Emergent Adverse Event
MDS-UPDRS	International Parkinson and Movement Disorder Society - sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS)
UPT	Urine Pregnancy Test
WOP	Wash Out Period

1. BACKGROUND

Parkinson's disease is a progressive neurodegenerative disorder caused by accumulation of the protein alpha-synuclein (αS) within the enteric nervous system (ENS), autonomic nerves and brain (Braak). The diagnosis of Parkinson's disease requires the presence of motor symptoms such as rigidity, bradykinesia and tremor (Hughes), although non-motor symptoms such as constipation (Ondo, Lin), disturbances in sleep architecture (Ondo, Gjerstad), depression (Aarsland), cognitive dysfunction (Auyeung) and hallucinations (Friedman, Diedirich) represent significant therapeutic challenges (Zahodne). While the motor symptoms are the result of dysfunction of dopaminergic neuronal circuits largely involving the *substantia nigra* and basal ganglia, non-motor symptoms largely result from impaired function of non-dopaminergic neural pathways. A strategy that targets neurotoxic aggregates of αS in the gastrointestinal tract represents a novel approach to the treatment of Parkinson's disease that may restore the function of the enteric nerve cells and prevent retrograde trafficking to the brain. Such actions could potentially benefit both dopaminergic and non-dopaminergic circuits and slow the progression of the disease in addition to restoring normal gastrointestinal function.

In 2003, Braak proposed that Parkinson's disease begins with the formation of toxic αS aggregates within the ENS and manifests clinically as constipation in a majority of people years before the onset of motor symptoms (Braak et al., 2006). We have recently reported that αS is induced in the ENS in response to viral, bacterial and fungal infections (Stolzenberg, et al., 2017) and that excessive intraneuronal accumulation of αS promotes formation of toxic aggregates (Perni et al., 2017). As a result of the normal trafficking of αS aggregates from the ENS to the central nervous system (CNS) via afferent nerves such as the vagus (Greene, 2014; Visanji et al., 2014), neurotoxic aggregates accumulate progressively within the brainstem and more rostral structures. Inhibiting αS aggregation in the ENS may reduce the continuing Parkinson's disease process in both the ENS and CNS (Phillips, et al., 2008).

ENT-01 is administered orally and acts locally on the ENS. The active ion of ENT-01, squalamine, an aminosterol originally isolated from the dogfish shark, has been shown to reverse gastrointestinal dysmotility in several mouse models of PD (West et al, manuscript in preparation). In addition, ENT-01 has been shown to inhibit the formation of aggregates of αS both *in vitro*, and in a *C. elegans* model of PD *in vivo*. In the *C. elegans* model in which the animal has been engineered to express human α-synuclein in its muscles, orally administered squalamine inhibits formation of membrane disruptive intracellular aggregates and produced a complete reversal of muscle paralysis (Perni et al., 2017).

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.

In a Phase 2a (RASMET) study involving 50 patients with Parkinson's disease and constipation, we assessed the safety of orally administered ENT-01, and the effect on bowel function and neurologic symptoms of PD. In addition, we identified a dose of ENT-01 that normalizes bowel function in each patient. The study achieved the objectives of identifying safety and pharmacodynamic responses and demonstrated that directly targeting αS pharmacologically can achieve beneficial GI, autonomic and CNS responses.

The effective dose ranged between 75mg and 250mg, with 82.4% of patients responding within this range. This dose correlated positively with constipation severity at baseline consistent with the hypothesis that gastrointestinal dysmotility in PD results from the progressive accumulation of αS in the ENS, and that ENT-01 can restore neuronal function by displacing αS and stimulating enteric neurons. These results demonstrate that the ENS in PD is not irreversibly damaged and can be restored to normal function.

Several exploratory endpoints were incorporated into the trial to evaluate the impact of ENT-01 on neurologic symptoms associated with PD. The MDS-UPDRS score, a global assessment of

motor and non-motor symptoms showed significant improvement. Improvement was also seen in the motor component. Improvements were also seen in cognitive function (MMSE scores), hallucinations, REM-behavior disorder (RBD) and sleep. Six of the patients enrolled had daily hallucinations or delusions and these improved or disappeared during treatment in five. RBD and total sleep time also improved progressively in a dose-dependent manner.

Most indices related to bowel function returned to baseline value by the end of the 2-week wash-out period while improvement in the CNS symptoms persisted. The rapid improvement in certain CNS symptoms is consistent with a mechanism whereby nerve impulses initiated from the ENS following ENT-01 administration augment afferent neural signaling to the CNS. This may stimulate the clearance of αS aggregates within the afferent neurons themselves as well as the secondary and tertiary neurons projecting rostrally within the CNS, since it is known that neural stimulation is accompanied by increased neuronal autophagic activity. We speculate that after cessation of ENT-01 administration, the neurons of the CNS would gradually re-accumulate an αS burden either locally or via trafficking from αS re-aggregation within the gut.

We are currently evaluating ENT-01 as a therapeutic to treat the constipation and neurologic symptoms associated with Parkinson's disease in a randomized Phase 2b (KARMET) study. Like the Phase 2a study, the randomized Phase 2b study has dosing between 75mg and 250mg and a treatment period limited to 25 days. This safety roll-over study is for patients who have completed the Phase 2b study, extending the treatment period for up to 14 weeks.

We recently completed a 19-week dog and 26-week rat study. There were no safety concerns specifically in relation to the GI tract.

Study rationale:

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. As a consequence, 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 (Macrogol) 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 involving 50 patients with Parkinson's disease and constipation was recently conducted (RASMET; IND 130770, NCT03047629). The purpose of the study was to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of 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 patients received escalating single doses from 25 to 200 mg/day or maximum tolerated dose (MTD). The goal was to determine safety and tolerability. In Stage 2, 34 patients received daily doses escalating from 75 to a maximum of 250mg/day, a dose that induced change in bowel function or MTD, followed by a "fixed" dose for 7 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% patients achieved the primary efficacy endpoint. Mean CSBM/week increased from 1.2 at baseline to 3.6 during fixed dose (p=1.2x10^{-7}) (Hauser et al., 2019). Common adverse events included nausea in 21/44 (47%) and diarrhea in 18/44 (40%) patients. No study-drug related serious adverse events were reported. The only SAE was a GI bleed after medication was discontinued in one patient. This patient was on aspirin, Plavix, and Motrin, and the SAE was considered unrelated to study medication. There were no deaths. The only other noteworthy AE was dizziness 8/44 (18%). Dizziness was graded as moderate in one patient who was receiving an alpha-adrenergic blocking agent (Terazosin). This patient was withdrawn from the study and recovered spontaneously. All other AEs resolved spontaneously without discontinuation of ENT-01. The details of the RASMET study AEs are in the study report contained in IND 130770.

Significant and lasting improvements were observed in total MDS-UPDRS scores (p=0.0008), the MDS-UPDRS-Part III scores (motor component) (p=0.0006), cognition (MMSE) (p=0.0004), hallucinations, sleep, and circadian rhythm (p=0.016). Hallucinations improved in 5/6 patients. 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.

A randomized, placebo-controlled Phase 2b study (KARMET) of orally administered ENT-01 is currently being conducted in patients with PD and constipation. Dosing is daily, beginning at either 75 mg (baseline CSBM 1.0-3.0) or 150 mg (baseline CSBM 0-0.9) and escalating by 25 mg every 3 days until pro-kinetic effect or a maximum dose of 250 mg. Total treatment duration is 25 days.

A top-line data cut of the first 57 patients (an extension study is currently being performed) showed that ENT-01 achieved the primary efficacy endpoint in the treatment of constipation, with CSBM frequency increasing from 1.0 at baseline to 4.8 at the end of the fixed dosing period. Six patients exhibited hallucinations and/or delusions at the beginning of the study. These neurological symptoms disappeared or improved in 5 subjects randomized to ENT-01 but not in the 1 placebo patient. The dose required for improvement or disappearance of these neurological symptoms was patient specific. There were also improvements in other CNS parameters, including motor symptoms and memory.

Many of the AE were confined to the GI tract and were mild to moderate. Common adverse events included nausea in 10/53 (18.3%), diarrhea in 4/53 (7.4%), and abdominal pain in 4/53 (7.4%). There were 4 SAEs, 2 hospitalizations for joint replacement (1 patient on ENT-01 and 1 on placebo), 1 hospitalization for cholecystectomy and 1 for diuretic induced hypokalemia with weakness. Two of the 6 falls in the ENT-01 group occurred during the treatment period, the other

4 did not. In both cases the patient had a prior history of falls. The study is on-going, and a study report will be filed at the conclusion of the study.

We recently conducted studies in the rat administered ENT-01 orally up to 80 mg/kg/day for 26 weeks and found no significant drug-related systemic or local toxicity, establishing the NOAEL to be ≥ 80 mg/kg/day. The maximum daily dose of ENT-01 to be administered in the planned trial is 250 mg (3.1 mg/kg), reflecting a safety margin of > 26-fold. We also conducted studies in the dog administered ENT-01 orally up to 20 mg/kg/day for 19 weeks and found no significant drug-related systemic or local toxicity, establishing the NOAEL to be ≥ 20 mg/kg/day. The maximum daily dose of ENT-01 to be administered in the planned trial is 250 mg (3.1 mg/kg), providing a safety margin of > 6-fold.

In Stage 1 of RASMET a single oral dose of 200 mg of ENT-01 (114 mg/m^2) resulted in an AUC $_{0\text{-}16hr}$ of about 160 ng*hr/ml. At an intravenous dose of 130 mg/m^2, the AUC$_{0\text{-}24hr}$ is estimated to be about 100,000 ng*hr/ml, or about 1000-fold higher, resulting in an estimate of ENT-01 oral bioavailability of less than 0.3%. The MTD of intravenously administered squalamine (as judged over a 5-day period of continuous infusion) was estimated to be between 300-700mg/m^2, where the corresponding AUC$_{0\text{-}24hr}$ was between 200,000-400,000 hr*ng/ml, respectively, representing plasma exposure over 1,000-fold higher than that reached following a 200 mg oral dose of ENT-01. Since nausea and diarrhea limit the maximal oral dose of ENT-01 to about 250 mg unintentional overdosing is unlikely to occur.

The only difference between this study and both previous studies is the duration of dosing. Both prior studies were limited to total dosing of 25 days, whereas in this safety roll-over study, subjects will be dosed for 14 weeks. Those subjects who were randomized to ENT-01 during the preceding Phase 2b study (KARMET Stage 2 Extension subjects,) will have been dosed for an additional 25 days, bringing the total dosing period to 18 weeks. Both the 19-week dog study and human data from the RASMET and KARMET studies support the safety of ENT-01 via oral administration up to 250 mg/day for 14 weeks. With oral bioavailability of less than 0.3%, orally administered ENT-01 attains systemic concentrations several hundred-fold lower than reached in the IV studies. In the RASMET and ongoing KARMET trials most AEs were mild, confined to the GI tract, and spontaneously resolved without discontinuation of ENT-01.

Since the systemic absorption of ENT-01 in man is <0.3%, our principal safety concerns focused on the local toxicity to the GI tract. No toxicity of this type has been observed to date in either the RASMET or the ongoing KARMET trials. Local toxicity to the GI tract will continue to be the focus of the proposed study.

2. OBJECTIVES AND ENDPOINTS

2.1. Primary Safety Objective

To determine the safety and tolerability of orally administered ENT-01 for approximately 14 weeks in subjects with Parkinson's disease-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 (EKG).

During the dosing period, it is anticipated that no more than 2 subjects out of 50 (4.0%) will have an adverse event of grade 4 or 5 that is at least possibly related to ENT-01. 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, the study will be put on an immediate clinical hold. Safety Monitoring processes are identified in Section 10.14.

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 that is at least possibly attributable to ENT-01

Tolerability

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 (i.e., a Grade 2 or higher AE, see Table 2).
- Recurrent diarrhea defined as 7 episodes of diarrhea within 24 hours of taking study medication on 2 consecutive days (i.e., a Grade 3 or higher AE, see Table 2).
- Dizziness defined as lightheadedness or fainting on rising from lying to sitting or standing that requires non-urgent medical intervention within 24 hours of taking study medication. (i.e., a Grade 2 or higher AE see Table 2).

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

2.2. Primary Efficacy Objective and Endpoints

The primary efficacy objective of the study is to evaluate the effect of orally administered ENT-01 in improving constipation in subjects with Parkinson's disease-related constipation.

Primary Efficacy Endpoint

The primary efficacy endpoint of the study is to determine the change from baseline in weekly CSBM rate during the 12 weeks of the fixed dose period. Baseline weekly CSBM rate will be determined during the preceding randomized study.

2.3. Secondary Efficacy Objectives and Endpoints

The Secondary Efficacy Objectives of the study are:

- Change from baseline in the symptoms of Parkinson's disease
- Change from baseline in cognition
- Change from baseline in psychosis

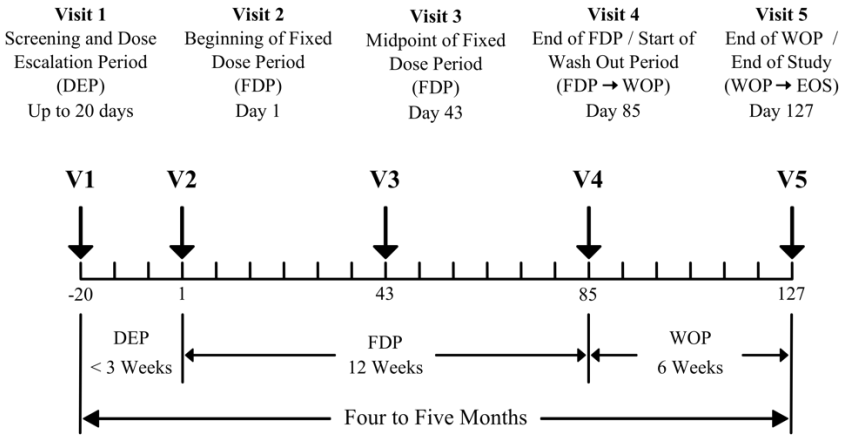
Secondary Efficacy Endpoints will include the following:

- Change from baseline in the MDS-UPDRS Parts 1-3 score at the end of the 12-week fixed dose period. Baseline is defined as the MDS-UPDRS score at the screening Visit of this roll-over study.
- Change from baseline in cognition will be assessed by MMSE scores at the end of the 12-week fixed dose period. Baseline is defined as the MMSE score at the screening Visit of this roll-over study.
- Change from baseline in psychosis as assessed by the SAPS-PD at the end of the 12-week fixed dose period. Baseline is defined as the SAPS-PD score at the screening Visit of this study.

3. STUDY DESCRIPTION

The purpose of this Study is to obtain safety data and stool diary data out to approximately 14 weeks (approximately 2 weeks of dose escalation plus 12 weeks of fixed dosing, see Figure 1 below.) All subjects who participated in the randomized KARMET trial (ENT-01-030, Stage 2 Extension subjects) and who are interested in participating in this open label safety roll-over study will be offered participation. Subjects from KARMET Stage 1 (non-randomized) are not eligible to participate in this roll-over study. There is no restriction on the time lag between the subject's prior participation in ENT-01-030 and eligibility to enroll in this study, and subjects may enroll in this study the same day as completing the final study visit (Visit 6) of ENT-01-030.

Figure 1: Illustration of Study Visits



Approximately 50 subjects will be entered at up to 20 sites in the US, assuming 75% of patients randomized to ENT-01 and 50% of patients randomized to placebo choose to participate. Each subject will have 5 visits to the clinic in person (under exceptional circumstances some of these may have to be conducted via Telemedicine): Visit 1/Screening or dosing initiation; Visit 2/Beginning of Fixed Dose period (FDP); Visit 3/End of 6 weeks of FDP; Visit 4/End of 12 weeks of FDP; Visit 5/end of 6 weeks of wash out period (WOP)/End of study. It is anticipated that the duration of study participation for each subject will be approximately 20 weeks, as it is anticipated that the majority of subjects will require less than 20 days to reach their fixed dose.

Subjects will discontinue opiates and all laxatives, bulking agents, stool softeners, suppositories, proton pump inhibitors, antacids, and certain antipsychotics and antidepressants from Visit 1/Screening until Visit 4/End of 12 weeks of FDP. Where on stable doses, subjects may continue the use of antihistamines and anti-histaminergic agents, anti-cholinergics, COMT inhibitors, dopamine agonists, amantadine, and certain anti-psychotic and anti-depressant medications. (See Section 7 Concomitant and Prohibited Medications and Therapies.)

Subjects will keep daily electronic stool diaries and will record information concerning stools immediately after each bowel movement, including stool frequency, consistency, ease of evacuation, completeness, and the use of rescue medication. Site staff will monitor these entries regularly to ensure subjects are not at risk of becoming impacted and are using rescue medication appropriately. Site staff will contact subjects if the web-based questionnaires are not being completed on a daily basis or if there are any safety concerns.

Each subject will be started on a pre-defined dose based upon their baseline CSBM rate during the randomized study, then will be able to increase their dose every 2-3 days until they reach a pro-kinetic dose or the maximum dose of 250mg. This is called the Dose Escalation Period (DEP). After the subject documents 4 CSBMs over 5 consecutive days, the subject will fix at that dose for the remainder of the study. This is called the Fixed Dose Period (FDP). At the end of the FDP medication will be discontinued and a 6-week wash-out period (WOP) will follow. Daily stool e-diary will be entered throughout the dosing period.

Rescue medications (including oral Bisacodyl, 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.

Study medication will be taken on an empty stomach first thing in the morning with 8 oz water and no food will be allowed for 60 minutes. Subjects prescribed levodopa should wait at least 30 minutes between taking levodopa and study medication. Tea and coffee with/without milk/sugar and orange juice (without pulp) are permitted. Plain yoghurt can be added if the patient experiences repeated nausea.

Symptoms of Parkinson's disease will be assessed using the Unified Parkinson's disease Rating Scale (MDS-UPDRS, Parts I-III) by certified raters and patients with fluctuations and prescribed levodopa will be assessed during "ON" states, approximately one hour after levodopa intake.

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 will be conducted at that time. (Early Termination visit assessments are the same as Visit 4.)

3.1. Pre-Screening Planning

Subjects who wish to continue taking ENT-01 after completing the ENT-01-030 study may "roll over" into this study. They can do this on the same day as their final study visit (Visit 6) for ENT-01-030, but they are not required to do so. Keep in mind the following to help with planning for "roll over" subjects:

- Informed Consent may be obtained before ENT-01-030 Visit 6 or Visit 1 of this study. Doing so would allow the subject to discontinue or refrain from the use of any prohibited medications listed in Section 7 prior to the visit, potentially allowing them to take the ENT-01 study medication earlier.
- There is no limit on the amount of time between completing ENT-01-030 and being able to screen for this study.
- If the subject "rolls over" within 14 days of ENT-01-030 Visit 6, then any procedures common to both studies do not need to be repeated. See Appendix 2: Schedule of Events and the individual visit descriptions below.
- Subjects may retain their ENT-01-030 diary device for use in this study.
- If the subject started or restarted any medications listed in Section 7 of this protocol before their ENT-01-030 Visit 6, they will need to discontinue them for 14 days before starting to take the ENT-01 study medication. This may affect the timing of Visit 2, depending on how long it takes them to reach a fixed dose.
- If a subject is taking levodopa and has fluctuations but does not "roll over" on the same day as their ENT-01-030 Visit 6, remind them about levodopa dose timing for the MDS-UPDRS.

3.2. Visit 1: Screening and Dose Escalation Period (Day -20 to Day 1)

Reminders

- Once consented and eligibility is confirmed, subjects with fluctuations and prescribed levodopa should take their daily levodopa dose so that they are in an ON state when the MDS-UPDRS is administered. For most subjects, this would be approximately 60 minutes after taking levodopa.
- Subjects must have discontinued and refrain from using any laxatives, constipation medications, and other medications as specified in Section 7 for 14 days prior to starting ENT-01 dosing. (These "washout" days are not counted in the schedule, as described below.)

Perform the assessments and procedures as listed in Appendix 2: Schedule of Events:

- Informed consent
- Inclusion/Exclusion Criteria
- Demographics/Medical History
- Complete Physical Exam
- Weight (Height from ENT-01-030 will be used, so it is not required to be recorded)
- Orthostatic Vital Signs: Vital signs will include supine blood pressure and heart rate, sitting or standing blood pressure and heart rate, respiratory rate, and body temperature. Supine measurements will be taken after the subject has been in the supine position for at least 5 minutes. Sitting or standing measurements will be taken within 5 minutes of the subject sitting or standing from the supine position.
- 12-lead EKG
- Labs (chemistry, hematology, and urinalysis): as defined in Appendix 1: Laboratory Tests.
- 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 of age, a serum FSH will be done at the screening visit only.
- Instruct subject to refrain from using or discontinue the use of any laxatives, constipation medications, and other medications as specified in Section 7 for 14 days prior to starting ENT-01 dosing. Determine the date ENT-01 dosing should begin and provide to the subject. (Although Visit 1 is listed as Day -20, that is more of a rough estimate for schedule planning. Most subjects should get to their fixed dose before 20 days; at that point Day 1 for that subject is set and their remaining visits are scheduled based on Day 1.)

- Dispense Rescue Medication and instruct on use: Subjects will be provided with rescue medications (including oral Bisacodyl, suppositories, and enemas,) and instructed on their use. Subjects will use rescue medications per investigator instructions to ensure adequate BMs every 3 days.
- Dispense ENT-01 with instructions regarding starting dose and escalation regimen.
- Educate subject with respect to stool diary daily entries.
- Assess for Adverse Events
- Review Prior and Concomitant Medications/Therapies
- MDS-UPDRS – Only Parts I, II, and III of the scale will be rated, assessed by personnel certified in the MDS-UPDRS. Subjects with fluctuations and prescribed levodopa should be assessed when in an ON state. Non-fluctuating subjects and subjects not on levodopa can be assessed at any point during the visit.
- SAPS-PD
- MMSE

The SAPS-PD is repeated at every visit only if the SAPS-PD score is > 0 at screening.

The purpose of the dose escalation is to achieve a pro-kinetic dose. The prokinetic dose is the dose that results in a CSBM on at least 4 of 5 consecutive dosing days. Starting dose will be determined by the baseline weekly CSBM rate as determined during the randomized study. Dose will be escalated by one tablet (25mg) every 2 or 3 days until a pro-kinetic dose or the maximum dose is reached. If repeated diarrhea occurs at a given dose, the dose will be reduced by one tablet (25mg). Care will be given to ensure that patients take rescue medication as instructed during this period.

Subjects will self-administer the study medication at home; either starting the following morning or on the date instructed. (The day the subject starts taking study medication is considered Day -20.)

- Subjects with baseline CSBM of 0-0.9 at the beginning of the randomized study will begin treatment at 150 mg of ENT-01.
- Subjects with baseline CSBMs of 1.0-3.0 at the beginning of the randomized study will begin treatment at 75 mg of ENT-01.

The dose will be taken upon awakening on an empty stomach along with 8 oz. of water. Subjects prescribed levodopa should wait at least 30 minutes between taking levodopa and study medication. The subject will be instructed to not eat for at least 60 minutes after study drug administration. Tea, coffee with/without milk/sugar and orange juice (without pulp) are permitted. Plain yoghurt can be added if the patient experiences repeated nausea. Dosing will be increased every 2-3 days until a pro-kinetic dose or the maximum dose is reached. If the subject has not

produced a CSBM within 24 hours of on at least 4 of 5 consecutive days, then they will increase their daily dose by 1 tablet (by 25 mg). This incremental dosing will continue until the subject:

- reaches the prokinetic dose (the dose producing a CSBM within 24 hours on at least 4 of 5 consecutive days). The subject then remains on that dose until Visit 4;
- reaches the maximum dose of 10 tablets (250mg for active treatment); or,
- reaches the dose limiting tolerability (DLT).

If the subject has 7 or more discrete episodes of diarrhea separated by 30 minutes or more within 24 hours at any dose on 2 consecutive days, the dose will be reduced by 25mg and fixed at that dose (assuming the diarrhea abates at the lower dose).

Should a prokinetic response not occur at the maximum allowed number of tablets (10 tablets or 250mg), subjects will continue to take 10 tablets throughout the Fixed Dose period.

Site staff will review subject diary entries daily and contact subjects as needed to ensure subject safety, compliance with study and dosing instructions, and to determine the subject's individual fixed dose level. The day that a subject "fixes" their dose for the first time (as determined by site staff) is considered Day 1 for the purposes of scheduling subsequent study visits.

3.3. Visit 2: Beginning of Fixed Dose Period (Day 1, +3 days)

This visit is to be done within 3 days from dose fixing. Perform the assessments and procedures as listed in Appendix 2: Schedule of Events:

- Brief Physical Exam (review of body systems as indicated)
- Weight
- Orthostatic Vital Signs: Vital signs will include supine blood pressure and heart rate, sitting or standing blood pressure and heart rate, respiratory rate, and body temperature. Supine measurements will be taken after the subject has been in the supine position for at least 5 minutes. Sitting or standing measurements will be taken within 5 minutes of the subject sitting or standing from the supine position.
- 12-lead EKG
- Labs (chemistry, hematology, urinalysis, pregnancy): as defined in Appendix 1: Laboratory Tests.
- IP Collection and Accountability
- Dispense Rescue Medication, as needed. Subjects continue appropriate use of rescue medication, ensuring adequate bowel movements every 3 days.
- Dispense ENT-01 for 6 weeks of dosing with instructions regarding dosing.
- Re-educate subject with respect to stool diary daily entries.
- Assess for Adverse Events (new, ongoing, since last visit, etc.)
- Review Concomitant Medications/Therapies (current, since last visit, etc.)
- MDS-UPDRS – Only Parts I, II, and III of the scale will be rated, assessed by personnel certified in the MDS-UPDRS. Subjects with fluctuations and prescribed levodopa should be assessed when in an ON state. Non-fluctuating subjects and subjects not on levodopa can be assessed at any point during the visit.
- SAPS-PD (for subjects who scored > 0 on the SAPS-PD at screening.)

3.4. Visit 3: 6th week of FDP (Day 43, +/- 3 days)

Perform the assessments and procedures as listed in Appendix 2: Schedule of Events:

- Brief Physical Exam (review of body systems as indicated)
- Weight
- Orthostatic Vital Signs: Vital signs will include supine blood pressure and heart rate, sitting or standing blood pressure and heart rate, respiratory rate, and body temperature. Supine measurements will be taken after the subject has been in the supine position for at least 5 minutes. Sitting or standing measurements will be taken within 5 minutes of the subject sitting or standing from the supine position.
- 12-lead EKG
- Labs (chemistry, hematology, urinalysis, pregnancy): as defined in Appendix 1: Laboratory Tests.
- IP Collection and Accountability
- Dispense Rescue Medication, as needed. Subjects continue appropriate use of rescue medication, ensuring adequate bowel movements every 3 days.
- Dispense ENT-01 for 6 weeks of dosing with instructions regarding dosing.
- Re-educate subject with respect to stool diary daily entries.
- Assess for Adverse Events (new, ongoing, since last visit, etc.)
- Review Concomitant Medications/Therapies (current, since last visit, etc.)
- MDS-UPDRS – Only Parts I, II, and III of the scale will be rated, assessed by personnel certified in the MDS-UPDRS. Subjects with fluctuations and prescribed levodopa should be assessed when in an ON state. Non-fluctuating subjects and subjects not on levodopa can be assessed at any point during the visit.
- SAPS-PD (subjects who scored > 0 on the SAPS-PD at screening)

3.5. Visit 4: 12th week of FDP, Start of WOP, ET (Day 85, +/- 3 days)

Perform the assessments and procedures as listed in Appendix 2: Schedule of Events:

- Brief Physical Exam (review of body systems as indicated)
- Weight
- Orthostatic Vital Signs: Vital signs will include supine blood pressure and heart rate, sitting or standing blood pressure and heart rate, respiratory rate, and body temperature. Supine measurements will be taken after the subject has been in the supine position for at least 5 minutes. Sitting or standing measurements will be taken within 5 minutes of the subject sitting or standing from the supine position.
- 12-lead EKG
- Labs (chemistry, hematology, urinalysis, pregnancy): as defined in Appendix 1: Laboratory Tests.
- IP Collection and Accountability
- Reinstate all discontinued medications, antacids, proton pump inhibitors and laxatives.
- Dispense Rescue Medication, as needed. Subjects continue appropriate use of rescue medication, ensuring adequate bowel movements every 3 days.
- Re-educate subject with respect to stool diary daily entries.
- Assess for Adverse Events (new, ongoing, since last visit, etc.)
- Review Concomitant Medications/Therapies (current, since last visit, etc.)
- MDS-UPDRS – Only Parts I, II, and III of the scale will be rated, assessed by personnel certified in the MDS-UPDRS. Subjects with fluctuations and prescribed levodopa should be assessed when in an ON state. Non-fluctuating subjects and subjects not on levodopa can be assessed at any point during the visit.
- SAPS-PD (for subjects who scored > 0 on the SAPS-PD at screening.)
- MMSE

3.6. Visit 5: End of 6-week Wash-out Period/End of study (Day 127, +/- 3 days)

Perform the assessments and procedures as listed in Appendix 2: Schedule of Events:

- Brief Physical Exam (review of body systems as indicated)
- Weight
- Orthostatic Vital Signs: Vital signs will include supine blood pressure and heart rate, sitting or standing blood pressure and heart rate, respiratory rate, and body temperature. Supine measurements will be taken after the subject has been in the supine position for at least 5 minutes. Sitting or standing measurements will be taken within 5 minutes of the subject sitting or standing from the supine position.
- 12-lead EKG
- Labs (chemistry, hematology, urinalysis, pregnancy): as defined in Appendix 1: Laboratory Tests.
- Assess for Adverse Events (new, ongoing, since last visit, etc.)
- Review Concomitant Medications/Therapies (current, since last visit, etc.)
- MDS-UPDRS – Only Parts I, II, and III of the scale will be rated, assessed by personnel certified in the MDS-UPDRS. Subjects with fluctuations and prescribed levodopa should be assessed when in an ON state. Non-fluctuating subjects and subjects not on levodopa can be assessed at any point during the visit.
- SAPS-PD (for subjects who scored > 0 on the SAPS-PD at screening.)
- Collect eDiary device.

4. STUDY POPULATION

Subjects will have participated in Stage 2 of the randomized, double-blinded KARMET study ENT-01-030. All subjects who complete the treatment period in that study will be offered participation in this safety roll-over study.

Number of Subjects

Approximately 50 subjects will be included, and approximately 40 will complete all visits, assuming a 20% early termination rate.

5. SELECTION CRITERIA

Subjects will be screened according to the inclusion/exclusion criteria below.

Inclusion Criteria

1. All subjects who participated in the randomized study ENT-01-030 (KARMET Stage 2 Extension subjects) and who completed the dosing period.
2. Subjects aged 30-90 years at the time of screening for the ENT-01-030 study, both genders.
3. Subjects must provide informed consent and be willing and able to comply with study procedures.
4. Subjects must be able to read, understand, and accurately record data into the diary to guarantee full participation in the study.
5. Female subjects must have negative serum or urine pregnancy tests and must not be lactating. For females able to bear children, a hormonal (i.e., oral, implantable, or injectable) and 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.
6. 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. Unable to withdraw proton pump inhibitors.
3. 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.
4. Diagnosis of secondary constipation beyond that of Parkinson's disease.
5. 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).
6. Neurological disorder other than Parkinson's disease that in the opinion of the investigator might interfere with the conduct of the study.

7. On treatment with intra-jejunal dopamine or carbidopa/levodopa (i.e. Duopa).
8. Subjects starting a new Parkinson's disease medication or modifying an existing medication within 2 weeks prior to enrollment.
9. Unable to maintain a stable diet regimen.
10. Subjects with a cognitive impairment that preclude them from understanding the informed consent.
11. Subjects placed under legal guardianship.
12. History of excessive alcohol use or substance abuse.
13. Any clinically significant abnormalities on screening laboratories or physical examination requiring further evaluation or treatment.
14. Females who are pregnant or breastfeeding.
15. Subject or caregiver unable to administer daily oral dosing of study drug.
16. Participation in a non-Enterin 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.

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 on the basis of 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 Visit 4 assessments as outlined in Appendix 2: Schedule of Events. The subject will be asked to return in 42 ± 3 days (6 weeks) to the site for Visit 5 as a safety follow-up visit.

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.

7. CONCOMITANT AND PROHIBITED MEDICATIONS AND THERAPIES

Categories of medications are listed below. Enterin will provide investigative sites with detailed listings of specific medications. All medications shall be reviewed and approved or disapproved by the investigator on a case-by-case basis.

Prohibited Medications to be discontinued at Screening (V1) and restarted during the wash-out period (V4):

- All oral laxatives, osmotic laxatives, pro-kinetic laxatives, stimulant laxatives, bulking agents, stool softeners, and suppositories, except for rescue medications used as directed in this protocol.
- Antacids
- Opiates
- Proton pump inhibitors
- Certain antipsychotics that worsen symptoms of Parkinson's disease.
- Certain antidepressants that worsen symptoms of Parkinson's disease.

Concomitant Medications permitted at stable doses

- Anticholinergics
- Antihistamines and anti-histaminergic agents
- Catechol-O-methyltransferase COMT inhibitors
- Certain antipsychotics
- Certain antidepressants, including tricyclic and quadricyclic antidepressants
- Dopamine agonists and amantadine (if on a stable dose for at least 2 weeks preceding the study).
- Certain dopamine reuptake inhibitors
- Dopamine metabolism inhibitors
- Dopaminergic compounds
- Selective serotonin reuptake inhibitors (SSRI)
- Serotonin and norepinephrine reuptake inhibitors (SNRI)

Permitted Therapies

- Deep brain stimulation, if present for more than 6 months and at stable stimulation parameters.

8. MATERIALS

8.1. Study Drug

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

8.2. Packaging and Labeling

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

8.3. Storage, Dispensing and Reconciliation of Study Drug and Identity of Investigational Products

All study medication should be stored at 20°C to 25°C (68°F to 77°F) until dispensed, at which time subjects may keep it at room temperature. 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 is the investigator's responsibility to ensure that accurate records of study medication issuance and return are maintained.

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

Table 1: Identification of Investigational Product

Product Name	**ENT-01**
Dosage form	Tablet
Dosage level (mg)	25 mg
Route/dosage	Oral. 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 minutes before breakfast with 8 oz. water

9. WARNINGS AND PRECAUTIONS

Subjects will be advised to stay at home for 4-6 hours after taking the first 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.

10. STUDY PROCEDURES

10.1. 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 Appendix 2: Schedule of Events.

10.2. Instructions to Subjects

Prior to entry into the study, subjects and their spouse/companion will be instructed about the use of rescue medication.

10.3. CTCAE Definitions of Dose Limiting Adverse Events

Table 2: NCI CTCAE v. 4.03 Definitions for DLT 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 a day over baseline	Increase of >7 stools a day; incontinence; hospitalization indicated	Life threatening complications: urgent intervention indicated	Death
Vomiting	1-2 episodes per 24 hours	3-5 episodes per 24 hours	6 or more episodes in 24 hours; tube feeding, TPN or hospitalization indicated	Life-threatening consequences: urgent intervention indicated	Death
Postural hypotension[3]	Mild, transient lightheadedness or dizziness upon rising from lying or sitting position that does not interfere with ADL	Moderate lightheadedness, dizziness or fainting upon rising from lying or sitting position that limits instrumental ADL; Non-urgent medical intervention indicated	Severe lightheadedness, dizziness or fainting upon rising from lying or sitting position that limits self-care ADL; Medical intervention or hospitalization indicated	Life-threatening and urgent intervention indicated	Death

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

10.4. Criteria for Dose Limiting Toxicity (DLT) Endpoints:

- Recurrent vomiting: 3-5 episodes of vomiting within 24 hours of taking study medication (Grade 2 adverse event)
- Recurrent diarrhea: >7 episodes of diarrhea within 24 hours of taking study medication (Grade 3 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 (Grade 2 adverse event) or either a systolic blood pressure less than 80mmHg or diastolic blood pressure less than 40mm Hg.

10.5. 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.

10.6. 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 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.

Separate summaries for adverse events that occur during treatment (summary of treatment emergent adverse events) and adverse events occurring during the wash-out period will be provided.

10.7. Laboratory Abnormalities

Clinical labs will be analyzed by a central laboratory. Labs to be drawn during the study include serum chemistries, a hematology panel, and urinalysis. On-site urine pregnancy testing is performed at each visit for women of child-bearing potential; positive tests are confirmed with a serum pregnancy test. For post-menopausal women less than 60 years of age, a serum FSH will be done at the screening visit only. Screening pregnancy tests must be negative prior to study entry. If any local laboratory is used, the investigator must obtain verification that it meets the standards for quality and consistency set by the College of American Pathologists.

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

- NS: Not clinically significant
- LE: Laboratory Error
- PT: 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. Refer to Section 10.9 through Section 10.12 for the definition of a serious adverse event and related terms, and for details on reporting a serious adverse event.

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

Table 3: Adverse Event Grading Terms

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 defined below:

Table 4: Adverse Event Relatedness Terms

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.

10.9. Reporting Requirements

Any adverse event, including both observed or volunteered problems, complaints, or symptoms that begins any time between the signing of the consent and within 30 days after the end of study participation 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, 21 CFR 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

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

10.10. Serious Adverse Events

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

10.11. Notification of Serious Adverse Events

Under IND regulations, 21 CFR Part 312.64, investigators must immediately report to the Sponsor any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

The Sponsor must be notified as detailed in Section 10.12, "Reporting a Serious Adverse Event." The Sponsor, in turn, will report all serious adverse events to regulatory agencies as required. In addition to the serious adverse events described in Section 10.10, 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.

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

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

10.12. 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 24 hours of knowledge of the event) by:

1. Entering the adverse event in the appropriate section (AE page and/or SAE page) of the eCRF, indicating that the event is considered serious, and providing all the details per the eCRF completion guidelines.

   AND

2. Completing the SAE Report Form and emailing or faxing to PAREXEL Safety Services.

In the event that the site is unable to complete the SAE form or eCRF entry to report the event within 24 hours of their knowledge of the event, investigators may report the SAE to PAREXEL Safety Services over the telephone. Then the eCRF must be completed or the SAE Report Form must be emailed within the following 24 hours.

Sites may obtain assistance with SAE reporting procedures or the specifics of reporting an event by calling PAREXEL Safety Services.

Serious Adverse Event Reporting		
PAREXEL Safety Services	Fax: 781-434-5957 Phone: 781-434-5010	NorthAmerica_Medical@parexel.com
Specific medical questions can be addressed to the Enterin Medical Director:		
William E. Gannon, Jr., MD	Phone: 703-447-2615	WGannon@capcitytek.com

The initial report should contain as much information as is available concerning the event in order to permit the Sponsor to file a report that satisfies regulatory requirements. Initial telephone reports of serious adverse events must be followed-up by completion of the AE/SAE eCRF or emailing the completed SAE Report Form as described above. When additional information is available, the investigator should provide an appropriate supplementary event narrative to PAREXEL Safety Services.

All appropriate SAEs will be reported to the appropriate regulatory authorities and central IRB by PAREXEL Safety Services within specified 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 local IRB where required.

10.13. 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.

10.14. Safety Monitoring

A Data Safety and Monitoring Board (DSMB) has been established to monitor the safety of the subjects during the study. The DSMB 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 methodology and the operating procedures for the safety reviews will be developed by the Chairperson in collaboration with the sponsor and will be documented in the DSMB Charter.

10.15. Stopping Rules

It is anticipated that no more than 2 subjects out of 50 (4.0 %) will have an AE of Grade 4 or Grade 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, the study will be put on an immediate clinical hold. Safety Monitoring processes are identified in Section 10.14.

In addition, individual safety stopping criteria will include:

- Reaching DLT before a prokinetic effect
- Serious Adverse Event at least possibly attributable to ENT-01.
- 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

10.16. 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.

10.17. 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.

11. DATA MANAGEMENT AND STATISTICS

11.1. 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 drug.
- **Fixed Dose Population:** The Fixed Dose Population will include all subjects who are in Safety Population who enter the Fixed Dose period of the study.
- **Efficacy Evaluable Population:** The Efficacy Evaluable Population will include all subjects in the ITT Population who complete their Fixed Dose period with no major protocol violations.

11.2. Analysis Methods

General Methods

Details of the statistical analysis methodology will be provided in a separate statistical analysis plan (SAP), which will be finalized prior to the interim analysis being performed.

Continuous variables will be summarized using the number of observations, number of observations above the limit of quantification (if applicable), mean, standard deviation (SD) median, and range. Categorical variables will be summarized using frequency counts and percentages.

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.

11.3. 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 v.4.03.

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 (Screening, dose escalation, Fixed Dose, Wash-Out). 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 experiences 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 (EKG)

EKG 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.

11.4. Tolerability Analyses

Tolerability will be measured by DLT endpoints as defined in Section 10.4. 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.

11.5. Efficacy Analyses

Primary Efficacy Endpoint Analyses

The primary efficacy endpoint is the change from KARMET study baseline in the weekly CSBM rate during 12 Weeks of the Fixed Dose Period. The primary efficacy endpoint analysis is a Mixed Model Repeated Measures (MMRM) analysis and will be performed on the Safety population. Sensitivity analyses for the primary efficacy endpoint will be specified in the SAP.

Secondary Efficacy Endpoint Analyses

The following secondary efficacy endpoints will be analyzed using the same methodology as the primary efficacy endpoint:

- Change from baseline in the MDS-UPDRS (Parts I-III) at the end of the Fixed Dose Period.
- Change from baseline in the MMSE at the end of the Fixed Dose Period.
- Change from baseline in the SAPS-PD at the end of the Fixed Dose Period.

11.6. Handling of Missing Data

The approaches to handling missing data will be specified in the SAP.

11.7. Sample Size/Power Considerations

To assess tolerability and safety, a sample size of 50 patients yields 92% probability that an AE with an underlying rate of 5% will occur in at least one of these patients.

12. ESTIMATED DURATION OF THE STUDY

This study has an estimated maximum duration of up to 20 weeks for each subject, as it is anticipated that the majority of subjects will require less than 20 days to reach their fixed dose. The study duration from the first subject in (FPFV) to the last subject out (LPLV) is expected to be approximately 17 months.

13. ADMINISTRATIVE PROCEDURES AND ETHICAL CONSIDERATIONS

13.1. 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.

13.2. Study Monitoring

During the study, a Clinical Research Associate (CRA) from Enterin, Inc. 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 eCRFs, and that investigational product accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the eCRFs 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 eCRFs 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 will be available between visits if the investigator(s) or other staff needs information or advice.

13.3. 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.

13.4. 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.

13.5. 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 and Enterin, Inc.’s policy on Bioethics.

13.6. 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), Enterin, Inc., 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.

13.7. 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 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 in the eCRF.

13.8. Amendments to the Protocol

Modifications to the protocol are only possible by approved protocol amendments authorized by the Enterin, Inc. 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.

13.9. Protocol Deviations

The protocol must be read thoroughly, and the instructions followed exactly. Enterin, Inc. will not grant waivers for protocol deviations. Any deviation to the protocol has to be reported as soon as possible to Clinical Research Associate/designee. The governing reporting guidelines for protocol deviations must be adhered to by the Investigator.

13.10. Study Termination

This study may be prematurely terminated, if in the opinion of the Investigator or Enterin Inc., there is sufficient reasonable cause. Written notification documenting the reason for study termination will be provided to the Investigator or EnterinInc.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 Inc.

13.11. 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.

13.12. Data Management

All data relating to study procedures will be entered on to eCRFs provided by Enterin, Inc. All forms must be completed electronically. All requested information must be entered in the eCRF. Sites will be trained on the eCRF Completion Guidelines which provide instructions on how to enter the required data in the Electronic Data Capture (EDC) system. Enterin, Inc. will provide the study sites with access and training to the Electronic Data Capture system.

eCRFs 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 designee, 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. The information collected from the daily sleep and stool diaries will be entered on an on-going basis. Any outstanding entries must be entered immediately after the final examination. An explanation should be given for all missing data.

13.13. Liability and Insurance

Enterin, Inc. 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.

13.14. 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 Enterin, Inc.

13.15. Data Quality Assurance

As per GCP guidelines, Enterin, Inc. 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 Enterin, Inc. of the request. Following this inspection and/or audit, the Investigator must notify Enterin, Inc. of any violation or deficiency noted by the regulatory authority.

14. 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.

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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)/Creatinine Ratio	Ketone Bodies
Red Blood Cell Distribution Width (RDW)	Calcium	Indicators of Blood and WBCs
Platelet Count	Carbon Dioxide	Specific Gravity
Differential - absolute and percent of:	Chloride	Urobilinogen
Neutrophils	Creatinine	
Lymphocytes	Glucose	
Monocytes	Potassium	
Eosinophils	Sodium	
Basophils	Total Bilirubin	
	Total Protein	
	Urea Nitrogen	
	Lactate Dehydrogenase (LDH)	
	Creatine Kinase, Total	

Pregnancy tests
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 of age, a serum FSH will be done at the screening visit only.

APPENDIX 2: SCHEDULE OF EVENTS

Period Name	Dose Escalation (DEP)	Fixed Dose (FDP)			Wash-Out (WOP)
Period Duration	**Up to 20 days**	**12 weeks**			**6 weeks**
Visit Number	**V1**	**V2**	**V3**	**V4**	**V5**
Study Day	**Day -20 to 1**	**Day 1**	**Day 43**	**Day 85**	**Day 127**
Visit Window	**N/A**	**+ 3 days**	**± 3 days**	**± 3 days**	**± 3 days**
Informed Consent	X				
Inclusion/exclusion	X				
Demographics	X				
Physical Exam	X	X	X	X	X
Weight	X	X	X	X	X
Orthostatic Vitals	X	X	X	X	X
EKG	X	X	X	X	X
Labs	X	X	X	X	X
Urine	X	X	X	X	X
Pregnancy Test	X	X	X	X	X
Discontinue Laxatives	X				
Restart Laxatives				X	
Adverse Events	X	X	X	X	X
Medications Review	X	X	X	X	X
MDS-UPDRS (Parts I-III)	X	X	X	X	X
SAPS-PD	X	X*	X*	X*	X*
MMSE	X			X	
Stool Diary Review	X	X	X	X	X**
Rescue Medications	X	X	X	X	
Investigational Product	X	X	X	X***	

* Conduct SAPS-PD only if greater than 0 at Visit 1.

** Collect Stool Diary device at V5 and do not return it to subject.

*** Investigational Product accountability only at V4; do not dispense.