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PROTOCOL TITLE

A Multicenter, Single-Dose, Multiple-Dose, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Orally Administered ENT-01 for the Treatment of Parkinson's Disease related Constipation. IND# 130770

PROTOCOL NUMBER

ENT-01

STUDY PHASE

Phase 1/2a

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STUDY SYNOPSIS

Study Title	Safety, Tolerability, Pharmacokinetic and Pharmacodynamic characteristics of single or multiple dose oral ENT-01 for Constipation in Parkinson's Disease.
Objectives	Stage 1: Primary Objective: Safety and Tolerability of single oral doses of ENT-01 in patients with Parkinson's Disease related constipation. Secondary objective: To evaluate the Pharmacokinetic (PK) characteristics of single oral doses of ENT-01 in patients with Parkinson's Disease and constipation.
	Stage 2: Primary objective: To determine the Safety, Tolerability, PK and Pharmacodynamic (PD) characteristics of repeated oral doses of ENT-01 in patients with Parkinson's Disease related constipation.
	Exploratory To determine the effectiveness of study drug in improving colonic motility. To collect information on the effect of study drug on Quality of Life Measures
Study Description	Multicenter, Single dose, Multiple dose safety, tolerability and dose-finding study leading into a Randomized, Double-blind, Placebo-controlled Study
Study Population	Parkinson's disease with constipation
Study Drug Administration	Oral
Study Procedures	Colonic motility will be determined by Sitzmarks method before and during treatment

Study Endpoints	Stage 1: Safety Endpoint: Adverse events as evaluated with patient report, vital signs, chemical chemistry and EKG Tolerability Endpoint: • Recurrent vomiting • Recurrent diarrhea • Abdominal pain • Postural hypotension/dizziness
	Stage 2: Primary Efficacy Endpoint: • Bowel function as determined by stool frequency
	Exploratory Endpoints • Colonic motility as determined by Sitzmarks test • Sleep diary • Skin temperature (I-Button)-assessed sleep parameters • Mood assessed with Beck depression inventory (BDI) • QOL, non-motor symptoms questionnaires
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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADL	Activities of Daily Living
AE	Adverse Event
BDI	Beck Depression Inventory
CIOMS	Council for International Organizations of Medical Sciences
CFR	Code of Federal Regulations
CRF	Case Report Form
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Tolerability
EMEA	European Agency for the Evaluation of Medicinal Products
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IB	Investigator's Brochure
ICF	Informed Consent Form
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	Intent to Treat
MMSE	Mini Mental State Examination
MTD	Maximum Tolerable Dose
NMSQ	Non-Motor Symptom Questionnaire
PAC-QOL	Patient Assessment of Constipation - Quality of Life
PAC-SYM	Patient Assessment of Constipation Symptoms
PDSS	Parkinson's Disease Sleep Scale
PFS	Parkinson's Disease Fatigue Scale
RBDQ	REM Sleep Behavior Disorder Questionnaire
SAE	Serious Adverse Event
TEAE	Treatment Emergent Adverse Event
ULN	Upper Limit of Normal
UM-PDHQ	University of Miami Parkinson's Disease Hallucination Questionnaire
UPDRS	Unified Parkinson Disease Rating Scale

WHO	World Health Organization

1 INTRODUCTION

Constipation is a common problem worldwide, affecting 2% to 27% of the population, with most estimates varying from 12% to 20% (Bouras and Tangalos, 2009). The prevalence of constipation increases to 30%-40% among people aged >65 years and women are disproportionately affected. In North America, 63M people meet the Rome IV criteria for constipation and in the US alone, constipation is responsible for over 2M physician visits annually. Laxatives are prescribed to 2-3M patients every year and furthermore, in most patients, the condition is chronic requiring life-long treatment (Bouras and Tangalos, 2009).

Constipation is much more common among patients with Parkinson's disease than in the general population. There are 1M people suffering from Parkinson's disease in the US, of which roughly 60%, or 600,000 suffer from chronic constipation and in most, the condition is chronic, severe and unresponsive to standard therapy (Ondo et al., 2012; Zangaglia et al., 2007). This represents an economic burden to the individual with Parkinson's disease and to the healthcare system. According to the most recent Federal Supply Schedule (FSS; April 2016), the average 30-day reimbursed price for a basket of orally administered drugs for constipation is approximately $260 or $3120 per year. This represents about $1.8B of prescription laxatives just for patients with Parkinson's disease.

Constipation not only constitutes a major economic burden but it also significantly affects the quality of life of the individual with Parkinson's disease, contributing to social isolation and depression. Furthermore, the severity of the symptoms correlates negatively with patient reported quality of life.

An effective pro-kinetic medication for individuals with Parkinson's disease would be a useful addition to the currently available treatments for this disabling condition.

1.1 Background

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

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

The individual with Parkinson's disease suffers from a form of constipation that is believed to be caused principally by delayed transit through the colon. In addition, defecation is often impaired by dysfunction of the Parkinson's disease patient's anorectal reflex. For many individuals bowel issues represent a significant detriment to quality of life. Failure to effectively manage this problem can also lead to bowel obstruction, especially as the terminal phase of Parkinson's disease approaches. A limited number of therapies have been subjected to clinical trials and they include agents that increase the fluid content of the stool, either by blocking fluid resorption or increasing the osmolar load within the intestine (Ondo et al., 2012; Zangaglia et al., 2007).

Squalamine is a small molecule, originally discovered in the liver of the dogfish shark, where it is delivered following a meal via the biliary tract into the intestine (Moore et al., 1993). When squalamine is applied to the isolated colon and/or jejunum of a mouse, the compound stimulates organized peristaltic waves (Kunze et al, manuscript in preparation). This effect occurs through direct stimulation by squalamine of the intrinsic primary afferent neurons (IPANS) which are the most abundant type of neuron within the ENS. The IPANs, in turn, excite the ganglia of the myenteric plexus, and promote propulsive peristalsis. In addition, intense electrical signals are directed to the brainstem via the vagus following exposure of the ENS to squalamine which persist for some time. Of relevance to this clinical study, squalamine reverses the impaired motility of the colon from aged animals engineered to express a Parkinson's disease phenotype.

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

1.2 Rationale for the Study

Constipation is a major clinical component of Parkinson's disease and is reported to occur in greater than 60% of affected individuals. The pathophysiological basis of constipation in Parkinson's disease is generally believed to be due to delayed transit through the colon (Edwards et al., 1991; Klingelhoefer and Reichmann, 2015). Several studies have demonstrated that transit of stool through the colon of an individual with Parkinson's disease is about 50% that measured in age matched controls. 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 (Magrogol) has been studied in a small placebo controlled clinical trial of individuals with mild constipation, and shown to provide benefit with respect to stool frequency and consistency (Zangaglia et al., 2007). A short term placebo controlled trial of Lubiprostone, a chloride channel activator which increases intestinal fluid secretion, was only effective in about 50% of those treated, and resulted in passage of loose stools/diarrhea in place of constipation (Ondo et al., 2012). Furthermore, Lubiprostone delays gastric emptying (Camilleri et al., 2006), a function already compromised in Parkinson's disease.

The pathophysiology of the 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 structural 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 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.

1.3 ENT-01 Dosing Rationale

ENT-01 is the phosphate salt of squalamine. For this study it has been formulated as a small 25 mg coated tablet. Dosing will range from 25mg to 200mg. The dose will be taken upon awakening on an empty stomach along with 8 oz. of water simultaneously to dopamine. The patient will not be allowed to ingest any food for at least 60 minutes after study medication. The compound is highly charged and will adsorb to foodstuffs, so it is being administered prior to feeding.

The dose rationale for this study was based on the extensive clinical experience gathered in the setting of clinical trials involving systemic administration of squalamine, experience gathered from its consumption in nutraceutical products, and pre-clinical pharmacology studies.

ENT-01, the phosphate salt of squalamine, is weakly soluble in water at neutral pH but readily dissolves at pH< 3.5 (the pH of gastric fluid). Squalamine, as the highly water soluble dilactate salt has been extensively studied in over 3 Phase 1 and 8 Phase 2 human clinical trials as an intravenous agent for the treatment of cancer and diabetic retinopathy (Bhargava et al., 2001; Connolly et al., 2006; Hao et al., 2003; Herbst et al., 2003). The compound is well tolerated in single and repeat intravenous administration, alone or in combination with other agents, to doses of at least 300mg/m^2). In the current clinical trial, ENT-01 will be administered orally to subjects with Parkinson's disease who have long standing constipation. Although this trial will be the first in man oral dosing study of ENT-01, humans have long been exposed to low doses of squalamine (milligram to microgram) in the various commercial dogfish shark liver extracts available as nutraceuticals (e.g., Squalamax). In addition, following systemic administration squalamine is cleared by the liver and excreted as the intact molecule (in mice) into the duodenum through the biliary tract. Drug related GI toxicology has not been reported in published clinical trials involving systemic administration of squalamine. ENT-01 has limited bioavailability in rats and dogs. Based on measurement of portal blood concentrations following oral dosing of radioactive ENT-01 to rat's absorption of ENT-01 from the intestine is low. As a consequence the principal focus of safety is on local effects on the gastrointestinal tract. However, ENT-01 appears to be well tolerated in both rats and dogs.

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

The proposed starting dose in the Stage 1 segment of the planned trial is 25 mg (0.33 mg/kg for a 75 kg subject). The NOAEL of a single oral dose in the rat is ≥ 1000 mg/kg, and in the dog, ≥200 mg/kg representing a relative safety margin for the proposed starting dose of about 3000x based on the rat and about 600x based on the dog. The proposed maximum single dose in Stage 1 is 200 mg (2.7 mg/kg for a 75 kg subject) is covered by a safety margin of about 300x based on the rat, and about 70x based on the dog.

The maximum proposed dose to be evaluated in Stage 2 of the planned trial is 175 mg/kg/day (2.3 mg/kg/day for a 75 kg subject), and the total daily dosing exposure will last no longer than 25 days. The NOAEL of 25 daily dosing in the rat is ≥ 80 mg/kg/day and in the dog, ≥ 20 mg/kg/day, representing a relative safety margin for the proposed maximum daily dose of at least 35x based on the rat, and at least 9x based on the dog.

No gastrointestinal lesions, either macroscopic or microscopic, were associated with oral dosing of ENT-01 any of the non-clinical studies conducted. Systemic blood levels of ENT-01 will be determined in this clinical trial. The chemical structure of squalamine (highly water soluble, amphipathic cationic zwitterion of molecular weight 628) predicts poor oral bioavailability. Squalamine is well tolerated when administered by continuous intravenous dosing at 200-300 mg/m^2 over days to months of 5 day continuous infusions. At these dosing rates steady state plasma concentrations range between 5-10 µg/ml. Reversible elevation of hepatic enzymes is the major dose limiting toxicity, observed at dose rates above 300 mg/ m^2, which is understood since squalamine is cleared by the liver.

The daily dosing range in the planned clinical trial is from 25 mg (14.7 mg/m^2) to 200 mg (114 mg/m^2). Oral dosing of ENT-01, because of its low oral bioavailability, is not anticipated to reach significant plasma concentrations in human subjects, based on nonclinical studies. Based on an oral bioavailability of 0.1% observed in both dogs and rats and the PK data from prior Phase 1 studies of IV administration, (where an IV dose of 12 mg/m^2 yielded a C_{max} of 0.8 µg/ml, and 90

mg/m^2, a C_{max} of 2 μg/ml) the maximum plasma concentrations in man following a 25mg oral dose would be on the order of about 0.5ng/ml, and following a 200mg oral dose, about 2ng/ml.

The principal uncertainty related to potential toxicology on oral dosing in humans is the local effect of ENT-01 on the GI tract. However, in both rats and dogs, oral dosing was not associated with any evidence of local damage to the GI tract, including the gastric mucosa, which experienced the highest direct concentration of ENT-01.

The principal adverse events we anticipate in human subjects will be those relating to the gastrointestinal tract, such as diarrhea, cramps, nausea, and vomiting. The trial is also designed to establish the pharmacodynamics of ENT-01 on the GI tract. These effects will be determined by correction of constipation, and by semi-quantitative Sitzmarks test of colonic motility.

From preclinical electrophysiological and motility studies conducted in the *ex vivo* mouse jejunum and colon, squalamine concentrations of between 30-100μM were required to stimulate the ENS and drive peristaltic contractions (Kunze et al, manuscript in preparation). Based on the known fluid volume of the fasting stomach in normal subjects after ingesting an 8 oz. glass of water (about 240 ml) (Mudie et al., 2014), a 100 mg dose of Enterin would achieve a concentration of about 500 μM in the stomach and would undergo progressive dilution as the bolus advanced rectally. If we assume that 100mg (about 60 mg/m^2) of ENT-01 is a prokinetic dose in Parkinson's disease patients, that the bioavailability of squalamine (the active moiety) is no greater than 1% then blood levels would correspond roughly to no greater than that observed after a 0.6 mg/m^2 i.v. dose (Bhargava et al., 2001; Hao et al., 2003; Herbst et al., 2003), which is at least 400-fold below the maximum tolerated intravenous dose of about 300mg/ m^2.

We will monitor for potential systemic adverse events after oral dosing of ENT-01, with our principal focus on the local effects of the compound in the GI tract.

2 OBJECTIVES

The purpose of this study is to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of orally administered ENT-01 at doses ranging from 25mg to 200mg in patients with constipation secondary to Parkinson's disease. Dosing will be individually titrated to tolerability and effect. A second objective of the study is to determine the effect of orally administered ENT-01 on gastric emptying and colonic motility. A third objective of the study is to collect information relating to sleep and other non-motor symptoms of PD.

2.1 Stage 1

Primary objective:

The primary objective of the Stage 1 study is to determine the safety and tolerability of a single oral dose of ENT-01 in patients with PD and constipation. The dose will be titrated to tolerability, starting at 25mg daily and increasing up to 200mg daily.

10 patients will be enrolled in the study for 7-12 weeks.

Safety end-point will be:

Adverse events as measured by patient report, vital signs, clinical chemistry, and EKG

Tolerability end-points (referred to as the dose-limiting tolerability (DLT)):

- Recurrent vomiting defined as 3-5 episodes of vomiting within 24 hours of taking drug
- Recurrent diarrhea defined as 4-6 episodes of diarrhea within 24 hours of taking drug
- Abdominal pain defined as moderate pain that limits instrumental activities of daily living (ADL) (e.g., preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.) within 24 hours of taking drug
- Postural hypotension defined as dizziness/lightheadedness or fainting on rising from lying to sitting or standing that limits instrumental ADL and severe enough to indicate non-urgent medical intervention within 24 hours of taking drug.
- A fall in systolic blood pressure to <80mm Hg upon rising from lying to sitting or standing
- A fall in diastolic blood pressure to <40mm Hg upon rising from lying to sitting or standing
- Elevation of LFTs > 3 times the upper limit of normal (ULN)
- A reduction of body weight of 10% or more

Secondary objective:

The secondary objective of the Stage 1 study is to evaluate the Pharmacokinetic (PK) characteristics of a single oral dose of ENT-01 in patients with PD and constipation.

Pharmacokinetics:

Animal studies have shown < 1% ENT-01 absorption following oral administration. Plasma samples will be obtained for pharmacokinetics before dose administration and at 1, 2, 4, 8, and 24 hours after dose administration on day 1 of each dose level. A PK analysis will be conducted if plasma concentrations exceed the lower limit of quantitation of 5 ng/ml using a validated analytical method (Li et al., 2004). The PK analysis of ENT-01 will be conducted by using model-independent methods as implemented in WinNonlin™. The PK variables analyzed will include $AUC_{0\text{-}24\ (ss)}$, $C_{max\ (ss)}$, $C_{min\ (ss)}$, $T_{max\ (ss)}$, CL/F, and λ_z,.

The PK variables are summarized as follows:

$C_{max\ (ss)}$	Maximum or peak measured plasma concentration
$C_{min\ (ss)}$	Minimum or trough measured plasma concentration
$T_{max\ (ss)}$	Time of maximum or peak measured plasma concentration
$t_{½z}$	Apparent terminal elimination half-life; calculated as ln(2)/ λ_z. The number of data points included in the regression will be determined by visual inspection, but a minimum of three data points in the terminal phase, excluding C_{max}, will be required to estimate λ_z.
$AUC_{0\text{-}24\ (ss)}$	Area under the concentration versus time curve over the 16-hour dosing interval; calculated using linear trapezoid rule
CL/F	Clearance defined as: Dose/$AUC_{0\text{-}24\ (ss)}$.

2.2 Stage 2

Primary objectives:

The primary objective of the Stage 2 study is to determine the safety, tolerability, pharmacokinetic and pharmacodynamic characteristics of repeated oral doses of ENT-01 in patients with Parkinson's disease and constipation.

Animal studies have shown that orally administered ENT-01 has significant pro-kinetic effect on *ex-vivo* colonic preparations and on *in-vivo* rats and dogs. The effect of orally

administered ENT-01 on bowel function in patients with PD and constipation will be determined clinically using stool frequency, consistency and ease of passage. Constipation will be defined according to history and Rome-IV criteria at baseline (See Inclusion Criteria). During the study, all participants will maintain a daily diary of stool frequency, consistency and ease of passage. Date and dose of study medication and of bowel movement, stool consistency according to the modified Bristol scale and ease of passage of each stool on a 7-point adjectival scale as well as use of manual aids and suppositories will be recorded.

Exploratory Objectives:

The first exploratory objective of the Stage 2 study is to determine the pro-kinetic effect of repeated oral doses of ENT-01 on colonic motility as determined by Sitzmarks capsule retention. The Sitzmarks test will be performed on days 1-5 of the two-week run-in period and again at the end of the randomized treatment period.

The exploratory objectives will be to collect information on the effect of orally administered ENT-01 on sleep, temperature, memory, energy and mood in patients with Parkinson's disease. During the study, all patients will maintain a daily sleep diary. They will record bed time, sleep time and morning waking, hours slept each night, number and duration of wakes, and leg twitching/thrashing during the night. In addition, we will evaluate sleep indirectly using an I-Button which measures skin temperature. Diurnal variations in temperature correlate well with the sleep-wake cycle (Sarabia et al., 2008; van Marken Lichtenbelt et al., 2006). The patients will be asked to wear the I-Button attached to a wrist-band for five consecutive 24 hour periods during the baseline period (Period 3.1), during the stable-dose period (Period 3.2), the randomized period (Period 4) and part of the wash-out period. They will remove it only during showering. Mood, fatigue, hallucinations, memory and executive function will all be assessed at baseline, during the fixed dose period and at the end of the randomization period using the Beck Depression Index (BDI), REM behavior disorder questionnaire (RBDQ), Parkinson Fatigue Scale (PFS-16), University Miami Parkinson Disease Hallucination Questionnaire (UM-PDHQ), NMS questionnaire (NMS), MMSE and Trail-making A and B), respectively.

Primary Efficacy End-point:

The primary efficacy end-point will be regularization of bowel function as determined by stool frequency. This will be defined as an increase in frequency by one or more spontaneous, complete bowel movement per week compared to baseline or three or more spontaneous, complete bowel movements per week. Assessment will occur at the end of the fixed-dose period (Period 3.2) and baseline will be obtained at the end of the run-in period (Period 3.1) and all patients (n=40) will be compared with their baseline bowel function. During the randomized period (Period 4) the treatment group will be compared to the placebo group using the same criteria (n=20 in each group).

Summary of success criteria: (at least one of the following is met)

- Complete spontaneous stool frequency increase by 1 or more over baseline or
- Spontaneous, complete stool frequency of three or more per week

Exploratory End-points:

The exploratory end-points include increase in colonic motility during the randomized period over baseline values and comparison of motility between treatment and control groups during the randomized period. Colonic motility will be measured by the mean colonic transit (MCT) time based on a single film estimate (Metcalf et al., 1987).

Other Exploratory End-points:

Sleep diaries will provide subjective assessment of sleep parameters. BDI will provide subjective assessment of mood. PFS-16, NMSQ and UM-PDHQ will provide subjective assessments of fatigue and hallucinations. Non-motor symptoms (NMS) questionnaire will provide subjective assessment of overall NMS burden and will be supplemented with the RBD and PDSS questionnaires. PAC-SYM and PAC-QOL will provide subjective assessment of bowel function and QOL issues. MMSE and Trail-making A and B will provide objective assessment of memory and executive function. The I-Button will provide objective assessments of skin temperature diurnal variations and will be a surrogate assessment of delays in sleep onset, awake periods and Circadian rhythm.

- Sleep diary
- I-Button

- BDI
- MMSE
- Trail-making A and B
- UM-PDHQ
- PFS-16
- NMSQ
- RBDQ
- PDSS
- PAC-QOL
- PAC-SYM

3 STUDY DESCRIPTION

The study will consist of 7 periods. Total duration will be 21-29 weeks (maximum treatment period for each individual is 4-8 weeks).

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

3.1 Periods

PERIOD 1 (Stage 1):

Run-in period for Sentinel Group (n=10)

Duration: 2 weeks

Conducted at home with 1visit to the clinical trial facility and a telephone call at day 14

Patients who meet the inclusion/exclusion criteria and who sign an informed consent will be enrolled in the study. The sequence of patients entering the study will be used to generate the patient identifying number for the conduct of the study (e.g., all case report forms, laboratory assessments). At the beginning of the run-in period, patients will be asked to stop benzodiazepines and other sedatives, hypnotics, opiates, proton pump inhibitors. Patients will remain off these medications throughout the study. They will be allowed to continue antihistamines, anti-cholinergics, anti-psychotics, dopamine, and anti-depressant medication at stable doses. Use of laxatives and suppositories will be documented in the stool diary. Laxatives, softeners, Dulcolax suppositories and enemas will be disallowed during the 24-hour period before the start of the treatment period. They will keep daily diaries and record stool frequency, consistency, ease of evacuation and use of suppositories, laxatives, softeners, fiber, chia or flax seeds, as well as a sleep diary and record various sleep parameters. At the end of the Period, they will be re-evaluated using the daily stool chart to assess the severity of constipation. ***A stool occurring within 24 hours of laxative, stool softener, bulking agent or suppository use will not be counted. Only spontaneous stools will be counted. A spontaneous stool is a stool occurring in the absence of a laxative, stool softener, bulking agent or suppository.***

Exploratory Sleep Assessment:

Between the 9th and 14th days (range 8-15 days), patients will wear an I-Button continuously except during periods of bathing (See Appendix). Measurement of extremity temperature has been validated against polysomnography as a surrogate for measuring sleep onset and fragmentation. Other baseline testing during this period will include NMSQ, PFS-16, BDI, UM-PDHQ, RBDQ, PDSS, MMSE, and Trail-making A and B, PAQ-SYM and PAC-QOL.

Symptoms of Parkinson's disease will be assessed using the Unified Parkinson's Disease Rating Scale (UPDRS) during ON states. ON-state will be reached one hour after dopamine intake.

Visit Day 1: Informed Consent

The eligible subjects will be asked to sign the informed consent after the study has been explained to them and after all their questions have been answered.

At the beginning of the run-in period, the sentinel group of 10 patients will have screening visits. In the screening visit inclusion and exclusion criteria will be accurately reviewed and constipation history assessed by detailed history about stool frequency, consistency, ease of evacuation, duration of constipation, use of laxatives, stool softeners, bulking agents and also using the Rome IV criteria; Patients with a history of constipation of longer than 6 months, with two or fewer spontaneous, complete bowel movements a week, unresponsive to milk of magnesia and requiring Miralax or another laxative or softener at least once a week will qualify. Patients will have a physical examination, their vitals will be checked and their past and present medical history as well as their medication intake will be reviewed. PAC-SYM and PAQ-QOL will be administered. Detailed information re smell, depression, anxiety, hallucinations, memory, swallowing, dyspepsia and sleep patterns will be collected. BDI, RBDQ, PDSS, MMSE, Trail-making A and B, NMSQ, UM-PDHQ and PFS-16 will be administered. Baseline blood chemistry will be checked. Patients with liver enzymes over 1.5x will be excluded from the study. Women with childbearing potential will have a negative pregnancy test to enter in the study and within 48 hours of each of the studies. They will also be instructed as to the use of daily diaries and I-Buttons and told to use them during a five-day period during the second week as described above and continue for the remainder of the study. The stool chart will contain daily information relating to bowel movement, stool consistency, ease of passage and use of laxatives, softeners, bulking agents and suppositories.

Phone call Day 14:

Assessment of constipation severity off medication

Participants will receive a phone call and be asked about adverse events. They will also provide their daily stool chart to assess the severity of the constipation and use of laxatives, softeners, bulking agents and suppositories. ***Patients with two or fewer complete, spontaneous bowel movements will qualify for the treatment period. A bowel movement occurring within 24 hours of suppository, softener, bulking agent or enema use will NOT be counted. A spontaneous stool is a stool occurring in the absence of a laxative, softener, bulking agent or suppository.*** PAC-SYM and PAC-QOL to be administrated on day 14, or at the clinic at Dose 1 visit the following day if they fail to bring the completed survey.

PERIOD 2.1 (Stage 1): Single dose

Safety, tolerability and pharmacokinetics of orally administered single-dose ENT-01 in Sentinel Group

Duration: 22-57 days

The 10 patients in the sentinel group will be assigned to Cohort 1 and will participate in 8 single dosing periods.

Subjects	D1	D4-8	D7-15	D10-22	D13-29	D16-36	D19-43	D22-50	D25-57
1-2	25mg	50mg	75mg	100mg	125mg	150mg	175mg	200mg	
3-4		25mg	50mg	75mg	100mg	125mg	150mg	175mg	200mg
5-6		25mg	50mg	75mg	100mg	125mg	150mg	175mg	200mg
7-8		25mg	50mg	75mg	100mg	125mg	150mg	175mg	200mg
9-10		25mg	50mg	75mg	100mg	125mg	150mg	175mg	200mg

All 10 patients will be pre-selected in advance of check-in for their first scheduled dosing period. All laxatives, suppositories, softeners, bulking agents and enemas will be disallowed during the 24-hour period before or after the administration of study medication. Patients will wear the I-Button continuously for the entire dosing period. The first two patients (subjects 1-2) will first be admitted and given a single dose. The study will be conducted at a hospital-based clinical unit and patients will be monitored for 24 hours after the dose. Unless there are any safety

concerns, patients will be released at this time and brought back for the next dosing on Day 4-8 (D4-8), after a minimum of a 72 hour observation period. Dopamine will be taken prior to the clinic visit on an empty stomach. Patients will self-administer the study medication during their clinic visit. The dose will be taken on an empty stomach along with 8 oz. of water after baseline observations in the clinic. The patient will not be allowed to ingest any food for at least 60 minutes after study medication.

Each dose period will be staggered, so that patients 1-2 will be administered a single dose of the drug at the lowest dose of 25mg. Once 24 hours have elapsed, and provided there are no safety concerns, they will be sent home and brought back on day 4-8 for the next dose. During the days the patients are home, they will complete the daily diaries and e-mail them to the study coordinators. The study coordinators will call the patients every other day starting on the day following discharge for each dose and determine with the patient if a suppository or laxative is needed, as well as inquiring about any adverse events. Diary information will also be discussed with the patients during the telephone calls. Patients 3-10 will be dosed after the first two patients have been observed for 72 hours, i.e. on Day 4. Patients 1-2 will also be brought back on Day 4-8 and will be given a single dose of 50mg. Once another 24 hours have elapsed and provided there are no safety concerns, they will all be sent home and instructed to return on Day 7 for the next dosing level. This single dosing regimen will be continued until each patient has been given a single dose of 200mg or has reached a dose limiting toxicity (DLT). DLT will be the dose which induces repeated vomiting, diarrhea, abdominal pain or symptomatic postural hypotension within 24 hours of dosing. **Maximum tolerated dose (MTD) will be the highest dose for which there was no more than 1 DLT endpoint** (refer to **Section 9.8**, DLT endpoint criteria)**.** A review of safety, tolerability and adverse events will occur prior to administration of each dose level and at the end of Period 2 with DSMB. After the last dose of medication, BDI, MMSE, Trail-making A and B, NMSQ, UM-PDHQ, PFS-16, RBDQ, PDSS, PAC-SYM and PAC-QOL will be administered.

Schematic for Stage 1, Period 2.1: Hypothetical dosing regimens

Pharmacokinetics: Timed blood samples (5.0 mL) will be collected into Li-Heparin treated tubes for analysis of ENT-01 plasma concentrations. Plasma samples will be obtained for pharmacokinetics before dose administration and at 1, 2, 4, 8, and 24 hours.

Once collected, PK samples will be stored in a -20ºC or -70ºC freezer until time of shipment. Labels and appropriate shipping materials will be provided to sites. The concentration of study drug will be determined from the plasma samples using a validated analytical method. Details of the method validation and sample analysis will be included with the final clinical study report.

Individual stopping criteria:

The complete panel of Blood Chemistries described in Appendix 1 are to be drawn 24 hours after each dose. Results will be reviewed the evening before the following dose. In addition, any patient with at least 1 DLT endpoint (see **Section 9.8**), or with another non-DLT gastrointestinal AE > grade 3 that is at least possibly attributable to ENT-01, or elevated AST, ALT, or total bilirubin > 3x ULN or a reduction of body weight of 10% or more from baseline will be terminated. ENT-01 has very little systemic absorption, and hence adverse events are expected to be within the gastrointestinal system.

Refer to **Section 9.12** for Adverse Event Grading and to **Section 9.8** for DLT Endpoint Criteria.

Go/No-go decision:

Safety: *We are assuming that no more than 1 patient out of ten (10%) will have an adverse event of grade 4 or 5 that is at least possibly related to ENT-01 in dose levels thought to be below therapeutic effect. Should there be more than 1 patient with AE grade 4 or 5 that is at least possibly related to ENT-01 for doses less than 200 mg, the study will be put on an immediate safety hold until an emergency DSMB meeting can be held to evaluate the study for possible discontinuation.*

Dose-finding: Beyond the safety and tolerability, this Period will allow us to determine the dose which is safe and tolerable in all 10 patients (STD) and which has a pro-kinetic effect in at least some patients (STDE: Safe and Tolerable dose in all and pro-kinetic Effect in at least some). We will start Period 3 with the STDE.

PERIOD 2.2 (Stage 1): Washout

Duration: 2 weeks

Patients will be observed off study drug and adverse events will be recorded. They will continue to keep a daily diary throughout the period. They will continue to wear the I-Button for five 24 hour periods between Days 1 and 5. During the second week, they will be restarted on all original medications but will continue their diaries until the termination of the study. They will be seen prior to study termination and BDI, MMSE, Trail making A and B, NMSQ, UM-PDHQ, PFS-16, RBDQ, PDSS, PAQ-SYM and PAC-QOL will be administered.

Visit 2: Day 14

Patients will be reviewed prior to discharge and any adverse events noted.

DSMB review of Stage 1: The DSMB will determine whether to proceed to Stage 2.

PERIOD 3.1 (Stage 2a)

Run-in period for Cohort 2 (n=40)

Duration: 2 weeks

Conducted at home.

Forty patients will be screened and included in the study on the assumption that roughly 15% will drop-out, leaving 34 patients who complete the study. Ten sentinel patients will have been pre-selected and ready to go before the study is initiated. Patients who fit the inclusion criteria and who agree to sign an informed consent will be included in the study. At the beginning of the run-in period, they will be asked to ***stop benzodiazepines and other sedatives, hypnotics, opiates, protein pump inhibitors and all laxatives, bulking agents, softeners and suppositories.*** They will be allowed to continue antihistamines, anti-cholinergics, anti-psychotics, dopamine and anti-depressant medication at stable dose and ***to use rescue medication for constipation as needed as long as they note it in the diary***. Patients will remain off these medications throughout the study. Patients who fulfill Rome IV criteria will be included in the run-in period. Patients will be required to be constipated for over 6 months, have two or fewer complete, spontaneous bowel movements per week, unresponsive to milk of magnesia and requiring Miralax or another laxative, bulking agent or softener at least once per week. ***Rescue medication for constipation will consist of 10mg suppository of Dulcolax, taken if no bowel movement has occurred for three consecutive days.*** Rescue medication will be taken on the fourth day in the morning. Dulcolax suppository can be repeated up to three times and will be followed by the use of an enema if the Dulcolax was ineffective. BDI, PFS-16, NMSQ, UM-PDHQ, RBDQ, PDSS, MMSE and Trail-making A and B, PAC-SYM and PAC-QOL will be administered. They will keep daily diaries and record stool frequency, consistency, ease of evacuation, completeness and use of suppositories (if it occurs) and various sleep parameters. The diaries will be reviewed daily by the study coordinator to ensure compliance. ***Patients with two or fewer complete, SPONTANEOUS bowel movements will have the Sitzmarks motility assessment, complete the run-in period and proceed to the treatment period. Bowel movements occurring within 24 hours of a suppository, (or accidentally used laxative, bulking agent, softener) or enema will not be counted.*** At the end of the first week, assuming they are allowed to proceed to the treatment period, they will also have Sitzmarks motility assessment to assess baseline colonic motility. Symptoms of Parkinson's disease will be assessed using the Unified Parkinson's Disease Rating Scale (UPDRS) during ON states. ON-state will be reached one hour after dopamine intake.

Sleep Assessment: Patients will wear an I-Button continuously from Day 8 of the Run-In Period to Day 5 of the Wash-Out period except during periods of bathing.

Visit Day 1: Informed Consent

The eligible volunteers will be asked to sign the informed consent after explanations of the study and after all their questions have been answered. Patients will be registered to the study after they have provided informed consent.

At the beginning of the run-in period, the new cohort of 40 patients will have a screening visit. In the screening visit inclusion and exclusion criteria will be accurately reviewed and constipation history assessed using the Rome IV criteria. Patients will be included who have been constipated for over 6 months, who have two or fewer complete spontaneous bowel movements per week unresponsive to milk of magnesia and requiring Miralax or another laxative, bulking agent, softener or suppository at least once weekly. ***Details relating to depression, anxiety, memory problems, hallucinations, loss of smell, voice difficulties and sleep patterns, thrashing and speaking out loud during sleep, swallowing difficulties and dyspepsia will be recorded.*** Patients will have a physical examination, their vitals will be checked and their past and present medical history as well as their medication intake will be reviewed. Baseline blood chemistry will be checked. Women with childbearing potential will have a negative pregnancy test to enter in the study and within 48 hours of each of the studies. After they have signed the consent form, participants will be scheduled for the study-days and entry into the randomization procedure. They will also be instructed as to the use of daily diaries and I-Buttons. BDI, PFS-16, NMSQ, PDHQ, RBDQ, PDSS, MMSE, UPDRS and Trail-making A and B, PAC-SYM and PAC-QOL will be administered. They will be given Sitzmarks capsules to take on Days 8, 9, and 10.

Day 8, Phone call Assessment of constipation severity off medication and of colonic transit

Daily stool diaries which they will have been filling in for a week will be reviewed and those ***patients with two or fewer complete, spontaneous bowel movements per week will proceed to Sitzmarks assessment.*** This will trigger shipment of study drug.

Sitzmarks procedure Days 8, 9, and 10:

- The patient will self-administer the Sitzmarks capsule (contains 24 radiopaque markers; Konsyl Pharmaceuticals, Texas, USA) by mouth with water on Days 8,9, and 10 and will consume their usual diet during this period. The patient will abstain from using suppositories or enemas until they return on Day 11. An abdominal XR will be scheduled on Day 11

Day 11 – Site Visit 2

An abdominal XR will be obtained to determine the location and the extent of elimination of the radiopaque markers. Patients who expel at least 80% (19 or more of the markers) have grossly normal colonic transit. If remaining markers are scattered about the colon, the condition is most likely hypomotility or colonic inertia. If remaining markers are accumulated in the rectum or rectosigmoid, the condition is most likely functional outlet delay. Radiographs will be collected in electronic format and subsequently analyzed in blinded manner by a centralized reader (Michael Camilleri MD, and Duane Burton, MHA, Division of Gastroenterology and Hepatology, Mayo Clinic). The patient will be given study medication (B) and a PAC-SYM and PAC-QOL to take home. This will trigger randomization to treatment or placebo and to PK or no PK.

Day 14- Phone call

Patients will be asked to complete the PAC+SYM and PAC-QOL questionnaires. They will be instructed to take the medication the following morning on an empty stomach and not to eat anything for at least 60 minutes thereafter. ***Baseline complete, spontaneous bowel movements (CSBM) will be calculated from the diaries at the 14 day phone call as CSBM/entries x 7.Baseline PAC-SYM and PAC-QOL and baseline ease of passage and consistency will also be obtained at this time-point.***

PERIOD 3.2 (Stage 2): Multiple doses

Safety, tolerability and pro-kinetic effect of orally administered multiple-dose ENT-01 in Sentinel Group followed by Remaining Cohort

Cohort 2 (40 patients, of which 10 comprise the sentinel group):

Duration: 17-19 days

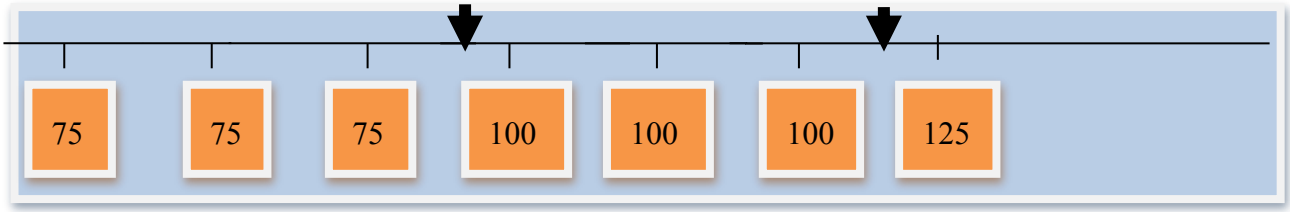
The purpose of this period is to demonstrate the continued pro-kinetic effect beyond that of a single-dose, to confirm the effective pro-kinetic dose for each individual and to establish the safety, tolerability and PK of multiple doses of ENT-01. ***A pro-kinetic dose is defined as the dose that results in a spontaneous, complete stool on at least two of the three dosing days.*** Once the pro-kinetic dose is established, and assuming there are no safety concerns, each patient will be

kept on that fixed dose for a further 2-4 dosing days (3 days if it falls on a Saturday, 4 days if it falls on a Sunday).

The sentinel group will be a group of 10 patients who did not participate in Stage 1. Patients will be given three consecutive doses of the dose which was found to be safe and tolerable in everybody (STDE) and effective in at least some patients in Stage 1 (75 mg in schematic) at home. Patients will self-administer the study medication ***daily*** for 3 doses (3 days at each dosing level). The dose will be taken upon awakening on an empty stomach along with 8 oz. of water simultaneously to dopamine). The patient will not be allowed to ingest any food for at least 60 minutes after study medication. The daily diary will be completed and results verbally transmitted and emailed to the study coordinator every evening including on week-end days and entered into the electronic data capture system the same night or first thing in the morning. The coordinator will check in with the patients daily, at the end of the day and determine whether there have been any adverse events or CSBM. If the patient has not produced a CSBM within 24 hours of a dose, on at least two of the three days at a given dose, then the patient will be told to proceed to the higher dose of STDE+25mg (eg.100mg qd) and also to use a suppository followed by an enema if necessary on the evening of the third day (See rescue diagram below). At the end of the second dosing period (100mg for 3 days), the same criteria will be applied to make a determination as to whether to proceed to STDE+50mg (e.g. 125 mg qd) or not. The incremental dosing will continue up to the consistently clinically effective (pro-kinetic) dose, or to the DLT, whichever comes first. Patients who do not have pro-kinetic effect at DLT will be treated with the highest dose at which they did not have a DLT, if it is at least the STDE dose; if the DLT occurred at the STDE dose, then the patient will be terminated. Should a pro-kinetic response not occur at 175 mg, patients will take 175 mg for the 2-4 additional fixed-dose dosing days and 6 randomized dosing days (assuming randomization is to treatment). DLT will be the dose inducing repeated vomiting or diarrhea within 24 hours of dosing. ***The lowest dose producing a CSBM on 2/3 consecutive doses will be noted as the pro-kinetic dose (LSTDEi i.e. consistently clinically effective safe and tolerable dose for that particular individual).*** Patients will be kept on the lowest dose producing a pro-kinetic effect for another 2-4 days prior to randomization in Period 4. The Enterin CMO will review the stool diaries prior to randomization to ensure dosing adequacy. PAC-QOL and PAC-SYM will be repeated prior to randomization at Visit 3. ***CSBM during the fixed dose period will***

be calculated as CSBM/#fixed dose days x 7. The primary end-point is obtained by comparing this number to the baseline CSBM obtained at the end of the run-in period.

2: SUPPOSITORY/ENEMA RESCUE USE DURING DOSE ESCALATION IN STAGE



Patients will wear I-Buttons starting with the lowest dose (e.g. 75 mg) and will remove them on day 5 of the wash-out period. The remaining 30 patients will start on STDE (e.g. 75mg) as soon as they have completed the Run-in Period and will follow the same incremental dosing regimen until they reach their LSTDEi or maximum tolerated dose. These regimens and adjustments are shown in the schematic below.

Once the LSTDEi has been established, each patient will remain on that dose for two to four additional days prior to randomization in Period 4 (5 to 7 consecutive days on prokinetic dose)

N.B. Primary end-point is obtained by comparing stool diary at this point with baseline diary on rescue medication at the end of the run-in period.

Visit 3 for non-PK patients (Day before Randomization).

Non- PK patients will come to the site for a visit to return any unused study drug (B), receive 6 daily doses of randomized drug (A or C), Sitzmarks capsules and will complete all the questionnaires listed below. They will be sent home with 6 daily doses of A or C and Sitzmarks capsules and will return for Visit 4 on Day 6 of randomization to complete all the questionnaires.

Go/No-go decisions:

Safety: *We are assuming that no more than 1 patients out of forty (2.5%) will have an adverse event of grade 4 or 5 that is at least possibly related to ENT-01 in this Stage. Should there be more than 1 patient with an AE grade 4 or 5 that is at least possibly related to Enterin-0 in this cohort*

of 40 patients, the study will be put on an immediate safety hold until an emergency DSMB meeting can be held to evaluate the study for possible discontinuation.

Tolerability: *We are assuming that all patients will exhibit a pro-kinetic effect at a dose lower than DLT and less than or equal to 175mg. Patients reaching DLT before a pro-kinetic effect will be terminated if the highest dose for the patient without a DLT was the STDE dose. Otherwise the patient will be treated at the highest dose at which they did not experience a DLT if they are randomized to the treatment arm (or will receive the equivalent dose of placebo).*

Efficacy: *We are assuming that a consistent pro-kinetic effect will be observed in all patients. Patients in whom a pro-kinetic effect is not observed at the maximum dose of 175mg will continue to take 175mg for another 2 days and enter the randomized period. If they are randomized to treatment, they will be given 175mg/day for the 6 randomized dosing days.*

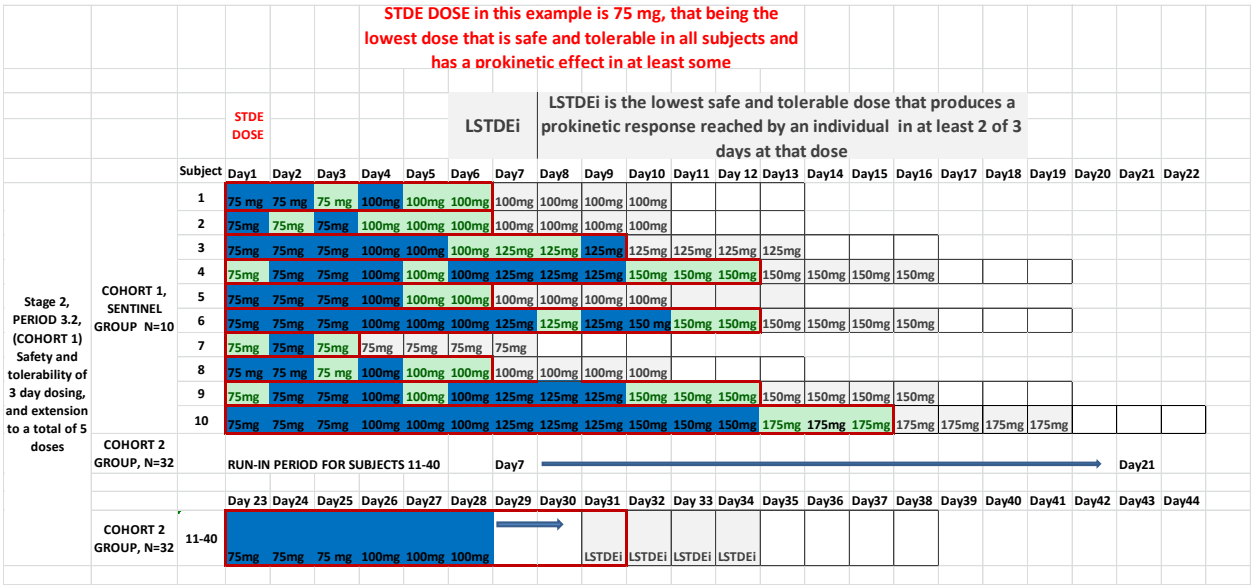
Individual stopping criteria:

Patients who reach DLT before a pro-kinetic effect, exhibit LFT elevation above 3xULN, have a 10% or greater reduction in body weight or those with a non-DLT gastrointestinal AE > grade 3 within 24 hours of taking ENT-01 that is at least possibly attributable to ENT-01 will be terminated.

Pharmacodynamics: Patient will record bowel movements, ease of passage, consistency and suppository usage in the daily diary throughout the Period and transmit the information to the coordinator every evening.

Sleep assessment: Patients will wear the I-Button throughout the dosing period and remove it only during bathing periods.

Schematic of Stage 2, Period 3.2 with hypothetical dosing regimen



DSMB: The DSMB will meet at the end of the fixed dose period for the ten sentinel patients.

PERIOD 4

(Stage 2): Randomized, double-blinded treatment period to determine the effect of orally administered ENT-01 on pharmacodynamics and motility.

Duration: 6 days

Visit 3 for PK patients (Day 1 of randomization):

PK subjects will come to the site on Day 1 of randomization ON AN EMPTY STOMACH and return their unused study drug at that time point. They will receive the first dose of medication at the site following the first PK blood draw, and complete the questionnaires listed below. PKs will also be performed at 1, 2, 4, and 8 hours post-dose. A sample for liver function tests will be drawn with the 8-hour post-dose PK draw. They will receive 4 daily doses to take home, then return on Day 6 to receive their 6th dose at the site after their first PK draw of the day. PKs will again be obtained post-dose at 1, 2, 4 and 8 hours, a sample for liver function tests will be drawn with the 8-hour post-dose PK draw, and all the questionnaires completed.

Following the 5-7 day course on the pro-kinetic dose, ALL patients will be randomized to drug or placebo and enter Period 4. Randomization will be 1:1 to treatment vs. placebo. Randomization will occur in accordance with the randomization schedule generated by Dr. Karla Ballman at Weill-Cornell Medical College. Patients and study staff will be blinded to actual study

treatment and will remain blinded throughout the remainder of the study. BDI, MMSE, Trail making A and B, NMSQ, UM-PDHQ, PFS-16, RBDQ, PDSS, PAC-SYM and PAC-QOL will be administered prior to randomization. The UPDRS will be administered and weight and vital signs will be taken).

Assuming randomization is to treatment group, the patient will remain on that dose daily for a 6 day period (6 dosing days). Patients randomized to placebo will be switched to the same number of placebo tablets as they were taking prior to switching. All patients will continue to keep daily diaries during this period. The use of rescue medication (suppositories and enemas) for constipation will be permitted as needed if a bowel movement has not occurred for 3 days and recorded in the diary. Patients will be contacted daily. The suppository will be taken on the evening of the third day as shown in Figure 1 above. ***Any bowel movement produced as a result of rescue medication will not be counted.***

Patients reaching their tolerability limit at a dose lower than a clinically effective dose (STDE) will be terminated from the study. We have included 40 patients in this Stage to allow for 15% drop-outs for reasons including tolerability.

Patients will wear the I-Button continuously throughout the randomized period except during bathing periods. Stool and sleep diaries will be completed daily throughout the period. BDI, MMSE, Trail making A and B, NMSQ, UM-PDHQ, PFS-16, RBDQ, PDSS, PAC-SYM and PAC-QOL will be administered on the first (Visit 3) and last day (Visit 4).

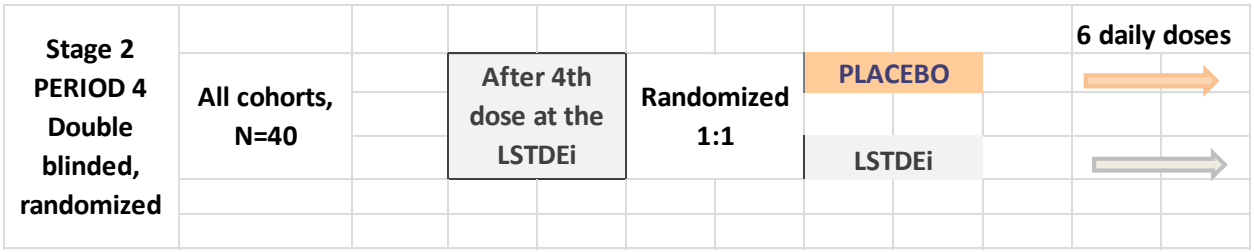
Pharmacodynamics WSW:

On Day 6 of this randomized period, patients will have a second set of motility tests (Sitzmarks capsules) to determine whether there has been any improvement in colonic motility. Patients will be compared against their own baseline motility and the placebo group will also be compared to the treatment group. The protocol will be identical to the baseline motility assessment. Patients will self-administer one capsule per day for three days beginning on Day 3 of the randomization period with subsequent capsules taken on Day 4 and Day 5. An abdominal XR will be scheduled on Day 6. Motility at the end of the randomized period will be compared with

baseline for all 40 patients and motility will also be compared between treatment and placebo groups (20 patients per group).

Safety and tolerability will be reassessed throughout the randomized treatment period. The study coordinator will call each patient at the end of each day and patients will be told to call the coordinator whenever they need to.

Summary of Stage 2, Period 4



Pharmacokinetics and LFTs: On the randomization Day 1, 7 patients from the treatment group and 3 patients from the control group will undergo blood draws for PK analysis at pre-dose, 1, 2, 4 and 8 hours after a dose and LFTs will be obtained on the last blood draw**.** These same 10 patients will undergo repeat blood draws for PK analysis and LFTs during their visit to the clinical trial unit on randomization Day 6 for the abdominal x-ray and repeated patient questionnaires/surveys. Study medication will be administered by study staff on randomization Day 1 and again on Day 6 after the first blood draw.

PERIOD 5 (Stage 2): Wash-out period

Duration: 2 weeks

Patients will be taken off study drug. They will continue to keep a daily diary throughout the period. They will be contacted by phone daily during Week One to check diaries and laxative use. During the first week of the wash-out period they will only use rescue medication as needed. During the second week, they will be restarted on all original medications including laxatives but will continue their diaries until the termination of the study. They will continue to wear the I-Button for the first 5 days of this period. During the last visit, the UPDRS, BDI, Trail-Making A and B, MMSE, PFS-16, NMSQ, UM-PDHQ, RBDQ, PDSS, PAC-SYM and PAC-QOL will be administered

4 STUDY POPULATION

Patients will be selected by neurologists specializing in movement disorders from among PD patients in tertiary referral centers. Patients will have PD and constipation as defined by the inclusion criteria. PD will be diagnosed according to the UK Parkinson's Disease Society Brain Bank criteria.

4.1 Number of Patients

Roughly 50 patients will be included. Ten patients will be included in Stage 1 and 40 patients in Stage 2. Assuming a 15% drop-out rate, 8-9/10 patients will complete Stage 1 and 34/40 patients will complete Stage 2.

4.2 Selection Criteria

Patients will be selected by individual site PIs from among PD patients seen at the tertiary referral center. Patients will be screened for the presence of non-motor symptoms of PD, specifically constipation and REM behavior disorder (RBD). ***The population will be enriched with respect to NMS, specifically sleep delay and sleep fragmentation problems, and RBD.*** The presence of constipation at baseline will be established according to the Rome IV criteria and other inclusion criteria below. Onset of constipation before or after a diagnosis of PD will specifically be noted and its duration and severity established. The daily stool diary will be analyzed at the end of the two week run-in period to establish the presence of severe constipation on rescue medication alone (does not apply to Stage 1). Patients with two or fewer complete, spontaneous bowel movements per week will be entered into the treatment phase. RBD will be determined by history and questionnaire. The use of anti-depressants, anti-cholinergics and anti-psychotics will be permitted as long as patients are on a stable dose of medication.

4.3 Inclusion Criteria

- Patients aged 30-86 year old, both genders
- Subjects must be diagnosed with Parkinson's disease (PD), according to the UK Parkinson's Disease Society Brain Bank criteria, by a neurologist specializing in movement disorders.

- There are insufficient criteria for IBS
- Constipation which has been present for over 6 months and is unresponsive to first line, typically over the counter treatments such as milk of magnesia (1 gram), Miralax (17g in 8 ounces of water) or the equivalent at least once weekly with an inconsistent response over a 6 week period.
- Body mass index of 18-40 kg/m^2
- Subjects must fulfill Rome IV criteria for functional constipation which includes 2 or more of the following:
    - Straining during at least 25% of defecations
    - Lumpy or hard stools in at least 25% of defecations
    - Sensation of incomplete evacuation for at least 25% of defecations
    - Sensation of anorectal obstruction/blockage for at least 25% of defecations
    - Manual maneuvers to facilitate at least 25% of defecations (e.g., digital evacuation, support of the pelvic floor)
- Fewer than 3 spontaneous, complete defecations a week.
- Loose stools are rarely present without the use of laxatives
- Patients must provide written informed consent and be willing and able to comply with study procedures.
- Patients must be able to read, understand, and accurately record data into the diary to guarantee full participation in the study.
- Female patients 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.
- Female patients unable to bear children must have this documented in the case report form (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

4.4 Exclusion Criteria

- Unable or unwilling to provide informed consent or to comply with study procedures.
- Diagnosis of secondary constipation beyond that of PD
- Structural or metabolic diseases that affect the GI system
- Functional GI disorder
- Unable to withdraw the following medications 2 weeks prior to the dose-escalation period and throughout the study: Laxatives, opiates, sedatives, hypnotics, protein pump inhibitors or any medications which may cause constipation
- History of recent major surgery (within 60 days of screening)
- Any clinically significant abnormalities on screening laboratories or physical examination requiring further evaluation or treatment.
- Neurological disorder other than PD
- On treatment with intra-jejunal dopamine or carbidopa/levodopa (i.e. Duopa)
- Treatment with COMT inhibitors (entacapone, tolcapone, Stalevo)
- Patients starting a new PD medication or modifying an existing medication within 4 weeks prior to enrollment
- Unable to maintain a stable diet regimen
- Patients with a cognitive impairment that preclude them from understanding the informed consent
- Patients placed under legal guardianship
- Acute GI illness within 2 weeks of the baseline period
- History of major GI surgery (e.g. previous abdominal surgery, including Cholecystectomy), except that patients with uncomplicated appendectomy are allowed.
- ALT or AST > 1.5 X upper limit of normal (ULN) during screening

- Females who are pregnant or breastfeeding
- History of excessive alcohol use or substance abuse
- Patient or caregiver unable to administer daily oral dosing.
- Participation in an investigational clinical study within the 6 months prior to dosing in the present study.
- Any other reason, which in the opinion of the Investigator would confound proper interpretation of the study.

4.5 Discontinuation Criteria

Patients may withdraw voluntarily from participation in the study at any time and for any reason. They may want to withdraw because of increased constipation off laxatives, nausea, vomiting or diarrhea.

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

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

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

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

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

4.6 Concomitant Medication

- Preventive laxatives will be discontinued during the run-in period. Throughout the study, patients will be allowed to take constipation ***rescue medication on demand***, and only if they have not had a bowel movement in three consecutive days.
- ***In stage 1***, ***rescue medication (laxative, bulking agent, softener, suppository)*** can be taken as needed and as stated above, and noted in the stool chart. Rescue medication cannot be taken during the 24 hrs before or after study medication. ***Any bowel movement produced within 24 hours of rescue medication will not be counted.***

In Stage 2, rescue medication (Dulcolax suppository and/or enema) can be taken on demand during the run-in period as stated above and noted in the stool diary. During the dosing period, rescue medication is taken on the evening of the third dose at any given level if a stool has not been produced in response to three days of study medication. Enterin CMO should be consulted for any questions related to rescue medication. Rescue medications will be recorded in the daily diary. ***Bowel movement produced within 24 hours of rescue medication will not be counted as either spontaneous bowel movement or complete spontaneous bowel movement.***

- In both stages, protein pump inhibitors will be discontinued during the run-in period and reinstated during the wash-out period.
- Benzodiazepines, hypnotics, other sedatives, barbiturates, and opiates will not be allowed. All medications shall be reviewed and dis/approved by the investigator on a case-by-case basis.
- Selective serotonin reuptake inhibitor (SSRI), SNRI, tricyclic antidepressants anti-cholinergics, anti-histamines and anti-psychotics are permissible at stable doses.
- Dopamine agonists and amantadine will be allowed if on a stable dose for at least 4 weeks preceding the study.
- Deep brain stimulation is allowed at the PI's discretion.

5 STRATIFICATION AND RANDOMIZATION (STAGE 2)

Participants will be randomly assigned to study drug or placebo in block sizes of 4 according to a schedule provided by the study statistician. **Patients will be stratified according to constipation severity. We will compare results in patients with average complete spontaneous stool frequency of 0-1 per week with those of patients with an average frequency of 1-2 per week.** Patients will be blinded throughout the study until all data are analyzed and locked.

6 UNBLINDING

Patients will be unblinded under the following circumstances:

- Patient is terminated from the study
- Patient drops-out of the study
- A SAE occurs

7 MATERIALS

7.1 Study Drug

All study medications will be supplied by Enterin Inc. Ent-01 will be supplied as 25mg tablets packaged in white opaque high density polyethylene (HDPE) bottles with child-resistant closures. The placebo tablets will be identical in configuration and packaged similarly.

7.2 Packaging and Labeling

The study medication will come packaged in a white bottle containing 10 tablets. Tablets can be divided between patients as appropriate. To ensure blinding the packages will be identified as A, B, or C, and each will contain 10 tablets, all equal in appearance.

7.3 Storage, Dispensing and Reconciliation of Study Drug

All study medication must be stored in a locked, limited access area. Both the used and unused study medication should be stored at 2 - 8°C (35.6 - 46.4°F).

Identity of Investigational Products		
Product Name	ENT-01	Placebo

Dosage form	Tablets (mg)	Tablet
Dosage levels(mg)	25	Identical, but absent active
Route/dosage	Oral. Doses above 25 mg will require multiple tablets: 25 mg=1 tablet 50 mg=2 tablets 75 mg=3 tablets 100 mg=4 tablets 125 mg=5 tablets 150mg=6 tablets 175mg=7 tablets	Oral
Dosing Instructions	Take 60 min before breakfast with 8 oz. water	Take 60 min before breakfast with 8 oz. water

8 WARNINGS AND PRECAUTIONS

Patients 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, patients themselves will be in a position to know if and when to leave the house.

9 STUDY PROCEDURES

9.1 Sitzmarks assessment of colonic motility in Stage 2

On Day 8 of the run-in period and again on *Day 3* of the randomized period, patients will be reminded to self-administer a Sitzmarks capsule (contains 24 radiopaque markers; Konsyl Pharmaceuticals, Texas, USA). The patient will be instructed to abstain from using suppositories or enemas if possible until they return on Day 11 (or day 6 of randomized period). If they have to use suppositories on day 11 (or Day 6 of randomized period), make sure it's recorded in the daily diaries. An abdominal XR will be scheduled for Day 11 of run-in and Day 6 of randomized period.

On Day 11 of the run-in Period and again on Day 6 of the randomized period, an abdominal XR will be performed to determine the location and the extent of elimination of the radioisotope markers. Patients who expel at least 80% (19 or more of the markers) have grossly normal colonic transit. If remaining markers are scattered about the colon, the condition is most likely hypomotility or colonic inertia. If remaining markers are accumulated in the rectum or

rectosigmoid, the condition is most likely functional outlet delay. For patients whose markers accumulate in the rectosigmoid or when markers are retained diffusely, instruct patient to maximize liquid intake for the next two weeks.

9.2 Observations and Measurements

All the questionnaires will be filled in according to the schedule. In Trail-Making B, the Trail will be completed twice, and both results will be reported. If the patient fails to complete the Trail, it will be reported as failed to complete.

Stool diaries and sleep diaries: Patients will be instructed how to fill the diaries daily throughout the study. They will be told to mark use of suppositories or enemas.

Vital signs: Per study procedures listings and for PK subjects Randomization Day 1 and Day 6 before dose and 2 hours after dose.

An I-Button which measures skin temperature and attached to a wrist band will be worn for 5 consecutive 24 hr periods from day 8-13 during Period 1, throughout the dosing period and randomized period and for the first five days of the wash-out period, and will not be removed except for brief periods during a bath/shower. Patients will be shown how to wear the I-Buttons with the button facing the skin and overlying the radial artery.

9.3 Informed Consent

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

9.4 Instructions to Patients

Prior to entry into the study, patients and their spouse/companion will be taught how to fill in the daily diaries for stool and sleep. They will be instructed about the use of rescue medication and told to record it in the diary. They will also be shown how to wear the I-Button and how to use the actigraphy device (See Appendix).

9.5 Clinical Laboratory Procedures

The following tests will be obtained at baseline and at the end of each Period.

- CBC
- Chemistry
- PT/PTT
- Pregnancy test
- Urinalysis

PK Samples for 10 selected subjects:

Timed blood samples (5.0 mL) will be collected into potassium-EDTA treated tubes for analysis of ENT-01 and its metabolite plasma concentrations.

Blood samples for PK analysis will be collected at the following times before and after dosing on the day before randomization and on Day 6 of the randomized period: 0 (pre-dose), 60 min, 2, 4, 8 hrs.

Once collected, PK samples will be stored in a -20ºC or -70ºC freezer until time of shipment. Labels and appropriate shipping materials will be provided to sites.

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

9.6 Other Procedures

Patients will fill in the following daily diary for stools (example inserted):

Date	**Dose mg**	**BM Yes/No**	**Consistency Modified Bristol Scale 1-7** 1.Hard lumps 2.Lumpy sausage 3.Cracked sausage	**Ease of passage scale 1-7** 1.Manual disimpaction 2.Enema needed 3.Straining needed 4.Normal	**Complete evacuation Yes/No**	**Suppository or laxative** **Yes/No/list med**

			4.Smooth sausage 5.Soft lumps 6.Mushy 7.Watery	5.Urgent without pain 6.Urgent with pain 7.Incontinent		
3/20	-	Yes	1	2	No	Yes/suppos
3/21	100	Yes	3	4	No	No
3/22	100	Yes	3	4	Yes	No
3/23	100	Yes	4	4	Yes	No
3/24	100	Yes	4	4	Yes	No
3/25	100	Yes	3	4	Yes	No

9.7 CTCAE Definitions of Dose Limiting Adverse Events

Adverse event	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Diarrhea (watery stool)	Increase of <4 stools a day over baseline	Increase of 4-6 stools 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 hrs	3-5 episodes per 24 hrs	6 or more episodes in 24 hrs; tube feeding, TPN or hospitalization indicated	Life-threatening consequences; urgent intervention indicated	Death
Abdominal pain	Mild pain	Moderate pain; limiting instrumental[1] ADL	Severe pain; limiting self-care ADL[2]	-	-
Postural hypotension[3]	Mild, transient lightheadedness or dizziness	Moderate lightheadedness, dizziness or	Severe lightheadedness, dizziness or	Life-threatening and urgent	Death

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

[1]*Preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.*
[2]*Bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden*
[3]*Definition adapted from combining CTCAE definitions for Hypotension and Dizziness*

9.8 Criteria for DLT Endpoints:

Recurrent vomiting: 3-5 episodes of vomiting within 24 hours of taking ENT-01 (Grade 2 AE)

Recurrent diarrhea: 4-6 episodes of diarrhea within 24 hours of taking ENT-01 (Grade 2 AE)

Abdominal pain: Moderate pain that limits instrumental activities of daily living within 24 hours of taking ENT-01 (Grade 2 AE)

Postural hypotension: Moderate dizziness, lightheadedness or fainting upon rising from lying to sitting or standing and severe enough to require medical intervention within 24 hours of taking ENT-01 (Grade 2 AE) or either a systolic blood pressure less than 80mmHg or diastolic blood pressure less than 40mm Hg.

9.9 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 patient exclusion criteria.

9.10 Treatment Emergent Adverse events

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

AE is typically collected after signing the informed consent form and could be related or unrelated to the study drug. TEAE is for after the subject actually taking the study drug.

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

9.11 Laboratory Abnormalities

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

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

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

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

If a laboratory value is considered to be serious and life-threatening the patient should be immediately discontinued from the study and appropriate therapy started. Refer to sections **9.7-9.12** for definition of a serious adverse event and related terms, and to sections **9.13-9.16** for details on reporting a serious adverse event.

9.12 AE Assessment and Recording

All adverse events, exacerbations of concomitant illnesses, or events known to be related to underlying disease processes or concomitant medications are to be recorded on the case report

form throughout the study. If a pre-existing condition worsens on study, the date on which the exacerbation began should be recorded. Onset dates for study treatment-related adverse events must be on or after the date of initial study treatment use. The need to record an adverse event on the case report form is not dependent on whether the adverse event is associated with the use of the study medication. In order to avoid vague, ambiguous or colloquial expressions, the adverse event 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. Patients with an adverse event should be carefully followed to determine outcome.

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

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

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

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

Grade 4: **Life-threatening consequences;** urgent intervention indicated

Grade 5: **Death**

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

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

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

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

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

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

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

9.13 Reporting Requirements

Any adverse event, including both observed or volunteered problems, complaints, or symptoms that begins any time between the start of the first dose and within 30 days after the end of the last dose are to be recorded briefly on the appropriate case report form and in detail in the source documents. A check list of adverse events may not be used during this study.

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

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

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

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

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

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

9.14 Serious Adverse Events

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

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

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

9.15 Notification of Serious Adverse Events

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

The Sponsor must be notified as detailed in section **9.16**, "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 **9.7**, other events that in the investigator's opinion suggest a significant hazard, contraindication, or precaution should be considered serious. This includes, but is not limited to, blood dyscrasias, endocrine disturbances, hemorrhage from any site, or severe skin disorder. Additional examples are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, and development of drug dependency or drug abuse. Hospitalization for elective surgery is not considered a serious adverse event. In addition, pregnancy is not a Serious Adverse Event, but is reportable and must be reported per IND regulations and timelines, 21 CFR Part 312.64, on the SAE reporting form and submitted to the sponsor.

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

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

9.16 Reporting a Serious Adverse Event

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

Safety@EnterinInc.com

and

Denise Barbut MD FRCP

denise@enterininc.com

d.barbut@enterininc.com

Cell 9179751377

And

Michael Zasloff MD PhD

M.zasloff@enterininc.com

Cell 484 433 7807

And

Heidi Marcus Moran

Hm.moran@enterininc.com

Cell 609.412.8411

Enterin Inc: Phone Number: 1-215-966-6083

Specific medical questions can be addressed to the Chief Medical Officer:

Denise Barbut, M.D, FRCP.

President and Chief Medical Officer

Phone (9179751377)

The back-up medical monitor, to be contacted in instances when the medical monitor is not available, is:

Michael Zasloff, MD, Ph.D

Chairman and CEO

Phone (484 433 7807)

The initial report should contain as much information as is available concerning the event in order to permit the Sponsor to file a report that satisfies regulatory requirements. Initial telephone reports of serious adverse events must be followed-up by a fax of a completed SAE

report form or an appropriate event narrative. The event should also be entered into the source documents and case report form, as appropriate. When additional information is available, the investigator should fax a follow-up SAE form or an appropriate supplementary event narrative to the Drug Safety Associate.

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

9.17 Serious Adverse Experiences: After Study Participation

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

- Any occurrence of a patient death is to be reported any time that the investigator becomes aware of the event
- A congenital anomaly in an offspring of a patient where that offspring has been born after the patient used study medications should be reported
- Any serious adverse event, for which, in the investigator's opinion, there is a reasonable possibility that the event could have been caused by the study drug, should be reported
- Any other serious adverse event should be reported if it occurred within thirty days following the last dose of study medication

9.18 Departure from Protocol for Emergency or Adverse Event

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

9.19 Data Safety and Monitoring Board

A Data Safety and Monitoring Committee (DSMB) has been established to monitor the safety of the subjects during the course of 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.

9.20 Review of Stage 1 Data by Data Safety Monitoring Board

When the last subject in Stage 1 has completed the course of dose escalation safety, PD and PK data will be reviewed by a DSMB, which will then provide a recommendation, based on guidelines established in the DSMB Charter, whether to advance to Stage 2. The starting dose for Stage 2 will be that dose which is safe and tolerable in all subjects and has a prokinetic effect in at least some ("STDE dose"). When the first ten subjects in Stage 2 have completed the course of dose escalation, data will be reviewed by the DSMB, which will then provide a recommendation, based on guidelines established by the DSMB Charter, whether to advance to the randomized controlled Period.

9.21 Stopping Rules

The DSMB will monitor the safety of the subjects during the course of the study. The DSMC can recommend changes to the protocol or temporary stopping of the trial at any time if there are significant safety concerns. The DSMC Charter will include recommended safety stopping rules.

9.22 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 case report forms which have not been monitored.

9.23 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 patient safety or the validity of the study must be approved by the IRB and submitted to the FDA before implementation. In the case of a medical emergency to increase safety of patients, a change may be made after discussion with the sponsor. In these instances the IRB and FDA will be notified as soon as possible.

10 DATA MANAGEMENT AND STATISTICS

This is a trial with two stages. The Stage 1 portion will consist of 10 patients who will be dose escalated in single doses. Each patient will proceed through all the dose escalations starting at 25 mg and ending at 200 mg or at the dose at which s/he experiences a dose limiting toxicity (DLT). The doses to be tested are 25mg, 50mg, 75mg, 100mg, 125mg, 150mg, 175mg and 200mg. In the Stage 2 portion, all patients will go through a multiple dose escalation (3 dosing days at each dose) and the dose at which the patient experiences a consistent pro-kinetic effect (spontaneous, complete stool in at least 2/3 consecutive doses) without a DLT will be the dose for that patient. Patients who experience a DLT prior to reaching their pro-kinetic dose and at a dose equal to or less than the STDE dose will be terminated. Otherwise the patient will be assigned the highest dose at which they did not experience a DLT. If the patient reached 175 mg without a DLT and without a pro-kinetic dose, her/his dose will be 175 mg. The primary endpoint for the Stage 1 study is to determine whether the dose levels explored are safe and tolerable. The primary endpoint for the Stage 2 study is the regularization of bowel function as determined by stool frequency at the end of the fixed dose period compared to baseline. The Stage 2 portion will enroll 40 patients, with a target sample size of 34 evaluable patients. Six extra patients are enrolled to allow for patient withdrawals or terminations prior to the completion of the trial.

10.1 Sample Size/Power Considerations

10.1.1 Stage 1

There will be 10 patients enrolled in Stage 1. Two patients will be started on the 25mg dose. If no DLTs are observed, they will be escalated to the next dose and the remaining 8 patients will be started at 25mg. Each dose level will be a single dose and patients will be observed for three days before being given the next higher single dose. It is intended that each patient receive a single dose of each of the levels: 25mg, 50mg, 75mg, 100mg, 125mg, 150mg, 175mg and 200mg. However, if a patient experiences a DLT, they will not be further escalated.

A DLT is defined in Section **9.8** using the Common Terminology Criteria for Adverse Events (CTCAE), as a grade 2 vomiting within 24 hours of taking ENT-01 grade 2 diarrhea within 24 hours of taking ENT-01, grade 2 abdominal pain within 24 hours of taking ENT-01, grade 2 postural hypotension within 24 hours of taking ENT-01. Additionally, an LFT elevation > 3xULN within 24 hours of taking ENT or any gastrointestinal AE > grade 3 within 24 hours of taking ENT-01 that is at least possibly attributable to ENT-01 will be considered dose-limiting toxicity for the purpose of analyses.

There is no power calculation for the Stage 1 portion because it is merely trying to determine whether ENT-01 is safe and tolerable for these levels. The PK analyses will be descriptive and are meant to understand how ENT-01 is metabolized.

10.1.2 Stage 2

The target sample size for Stage 2 is 34 evaluable patients and an additional 6 patients will be accrued to allow for patient drop-outs and terminations; these 40 patients will be different from the 10 patients accrued to Stage 1. A patient is evaluable if s/he has baseline measurements (obtained at the end of the run-in Period, 3.1) and measurements at the end of the fixed dose period (end of Period 3.2). The primary endpoint for Stage 2 is average complete spontaneous stool frequency increase by 1 point over baseline, OR 3 or more complete spontaneous stools per week. A patient will be labeled as a "success" if s/he meets one or more of the criteria above, otherwise the patient will be labeled as a "failure". The primary analysis compares the proportion of patients deemed a success at the end of the fixed-dose period (end of period 3.2) to 0.10, which is the highest proportion of spontaneous resolution of constipation assuming no treatment. A patient is deemed a success or failure by comparing the assessment at the end of the fixed-dose period (Period 3.2) to baseline (obtained at the end of the run-in period, Period 3.1). There could be between 34 and 40 patients with these measurements and each patient will be labeled as a "success" or "failure". It is assumed that without treatment, the success rate would be 10% (null hypothesis). An exact binomial test with a 0.05 two-sided significance level will have 80% power to detect the difference between 0.10 (proportion expected if patients are not treated), p_0, and an ENT-01 proportion, p_E, of 0.29 (0.27) when the sample size is 34 (40). It is assumed that most patients will be deemed a success on the ENT-01 arm and so the study is adequately powered.

10.2 Data Collection and Case Report Form Monitoring

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

10.3 Endpoint definitions and measurements

10.3.1 Assessment of Safety

Safety assessments (vital signs, physical examinations, interim medical history, vital signs, EKG (when clinically warranted for any unscheduled EKG), AEs, clinical laboratory results (routine hematology and biochemistry) are to be performed at protocol-specified visits, as specified in the Schedule of Procedures and Assessments.

Vital Signs

Vital signs (body temperature, respiration rate, heart rate, systolic and diastolic blood pressure measurements) will be evaluated at the visits indicated in the Schedule of Procedures and Assessments. Blood pressure will be taken twice. The first time, the patient shall be lying down. The second blood pressure will be taken within 5 minutes of sitting or standing from the recumbent position. All other vital signs will be measured after the patient has been resting in a sitting position for at least 5 minutes. Blood pressure measurements are to be taken in the same arm for the duration of the study. Body weight (without shoes) will be recorded whenever vital signs are recorded, and height (without shoes) will be recorded at screening only.

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

Physical Examination

A complete physical examination (head, eyes, ears, nose and throat, heart, lungs, abdomen, skin, cervical and axillary lymph nodes, neurological, and musculoskeletal systems) will be performed at Visit 1. Physical examinations will be performed by a physician. In addition, medical history will be recorded at screening, including smoking history, if applicable.

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

Electrocardiogram (EKG)

A 12-lead resting EKG will be obtained at the visits indicated in the Schedule of Procedures and Assessments.

An assessment of normal or abnormal will be recorded and if the EKG is considered abnormal, the abnormality will be documented on the CRF. EKGs will be repeated if clinically significant abnormalities are observed or artifacts are present.

Laboratory Assessments

Laboratory assessment samples are to be obtained at designated visits as detailed in the Schedule of Procedures and Assessments.

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

Blood and urine samples will be analyzed at the laboratory facility at the Investigational site. Urine samples will be analyzed by dipstick, and a microscopic analysis will be performed if the results of dipstick indicate abnormalities to be further investigated. All laboratory reports must be reviewed, signed, and dated by the Investigator. A legible copy of all reports must be filed with both the patient's CRF and medical record (source document) for that visit. Any laboratory test result considered by the Investigator to be clinically significant should be considered an AE (clinically significant AEs include those that require an intervention). Clinically significant abnormal values occurring during the study will be followed until repeat test results return to normal, stabilize, or are no longer clinically significant.

Adverse events

All reported AEs will be coded using the CTCAE version 4.03 terminology. The incidence of treatment-emergent AEs (TEAEs; events with onset dates on or after the start of the study drug) and treatment-related AEs will be summarized by system organ class (SOC) and preferred term

(PT). Events with missing onset dates will be included as treatment-emergent. If a patient experiences more than 1 occurrence of the same AE, the occurrence with the greatest severity and the closest association with the study drug will be used in the summary tables. SAEs and AEs that result in treatment discontinuation will be summarized. In addition, AEs of special interest will be summarized by SOC and PT; categories of AEs of special interest include: nausea, vomiting, abdominal pain, diarrhea, and rectal bleeding.

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

10.3.2 Efficacy Endpoints

Frequency of bowel movements:

The frequency of bowel movements will be determined via a daily diary where the passage of a bowel movement or absence thereof will be recorded daily throughout the study. ***Bowel movements that are the consequence of a rescue medication or suppository will not be counted if they occur within 24 hours of the rescue medication or suppository.*** Episodes of incontinence will also be recorded.

Success will be defined as an average complete spontaneous stool frequency increase by 1/week or more compared to baseline or 3 or more complete spontaneous stools/week. Baseline will be the run-in period weekly average and treatment period will be determined as the weekly average during the fixed dose (40 patients) treatment period.

Colonic Motility:

Colonic motility will be determined using the Sitzmarks technique at the beginning of the run-in period and again at the end of the randomized treatment period during Stage 2. The primary measure of interest is the mean colonic transit (MCT) time estimated with a single film (Metcalf et al., 1987).

10.4 Analysis Plan

10.5 Stage 2

Primary analysis: The AE information for the Stage 2 portion of the trial will be summarized as frequency and relative frequency by each dose level and for the LSTDEi for each person.

The primary efficacy outcome variable is whether or not a patient is a "success" or "failure". This is an endpoint based on patient diary entries for the week prior to the endpoint assessment defined as average complete stool frequency increase by 1 or more over baseline, OR 3 or more complete spontaneous stools/week. The patient is deemed a "success" if s/he meets one or more of the criteria listed above, otherwise the patient will be deemed a "failure". The primary analysis will be based on all patients with a baseline assessment (the end of the run-in period, Period 3.1) and an assessment at the end of the fixed-dose period (Period 3.2) and will be a comparison of the proportion of successes with 0.10 (the null hypothesis corresponding to no treatment effect). The proportion of patients for whom the drug was a success at the end of period 3.2 will be estimated with a binomial point estimate and corresponding 95% confidence interval. The observed proportion of successes will be compared to 0.10 using an exact binomial test. ENT-01 will be deemed as demonstrating sufficient efficacy if the two-sided p-value is less than 0.05 for this comparison.

Secondary analyses:

A secondary analysis will compare the proportions of patients who are deemed a success at the end of the randomized period (Period 4) as compared to baseline (end of the run-in period, Period 3.1) between those randomized to the ENT-01 arm and those randomized to the placebo arm. Patients to be included in this analysis will be those who have a baseline assessment (end of the run-in period) and an assessment at the end of the randomization period of the trial, which could be between 34 and 40 patients (17 to 20 in each arm). A Fisher's exact test will be used to compare the proportions of patients who are deemed a success at the end of randomization period (period 4) between the two randomized arms. Another secondary analysis will compare the proportions of patients who are a success (compared to baseline) between the end of the first week of the washout period (period 5) to the end of the randomization period (period 4), for those

patients who were randomized to receive ENT-01 (between 17 and 20 patients). This analysis will consist only of those patients with assessments at baseline (end of run-in period, Period 3.1), the end of Period 4, and the end of Period 5. This analysis will have between 17 and 20 patients and will be done using an exact sign test of equality of paired proportions.

Exploratory analyses:

The first exploratory analysis will of the colonic motility values as measured by an estimate of the mean MCT times. Comparisons will be made within each patient at different time points (i.e. baseline and the end of fixed period) as well as between patient groups (i.e. values observed at the end of randomization period, period 4 for the placebo and the ENT-01 treated groups). The primary endpoint for this analysis is the MCT based on a single film estimate. From a previous study (Ashraf et al., 1997), and estimate for the average MCT for constipated patients with Parkinson's disease is 53.4 hours and an estimate of the standard deviation is 15.9 hours. A paired t-test will be used to compare the mean change in MCT at the end of the fixed period from baseline. A sample size of 34 (40) patients will have 80% power to detect a mean change of 11.1 hours (10.2 hours) with a 0.05 two-sided level of significance and using a paired t-test. This calculation assumed a conservative estimate of 22.4 hours for the standard deviation of the change in MCT, which is the square root of 2 times the variance of the baseline standard deviation. The comparison of the average MCT between the control group and the group randomized to ENT-01 at the end of the randomization period will be made with a two-group t-test.

Exploratory analyses will also be done with respect to the sleep data, the body temperature data, mood, fatigue, hallucinations, overall NMS scale and all other questionnaires and neuro-cognitive tests. These parameters will be summarized numerically and graphically. There will also be comparisons done of changes over time within a patient (i.e. between baseline, end run-in period, and at the end of the fixed dose period, Period 3.2) and differences between groups (i.e. between patients in the Enterin arm and in the placebo arm at the end of period 4). As before, continuous measurements within a patient will be compared with a paired t-test (transforming the data if necessary) and continuous measurements between patient groups will be compared with a two-group t-test (transforming the data if necessary). In addition, these data will be analyzed using a mixed linear model since there are multiple measurements over time. Categorical data will be

compared with a chi-squared test or a Fisher's exact test if the expected cell counts are too small for a chi-squared test.

11 ESTIMATED DURATION OF THE STUDY

This study has an estimated duration of 8 weeks for each patient during each stage. Maximum dosing days for each patient will be no more than 8 days during Stage 1 and 25 days during Stage 2. The study duration from first patient in to last patient out is expected to be 9-12 months.

12 ADMINISTRATIVE PROCEDURES AND ETHICAL CONSIDERATIONS

12.1 Medical Care

This study has an estimated duration of 21 weeks. During this period, patients will be on medication for a maximum of eight weeks. None of the patients will be given medication during the run-in period or the wash-out period.

12.2 Patient Information and Informed Consent

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

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

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

12.3 Study Monitoring

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

- Determine the adequacy of the facilities

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

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

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

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

12.4 Audits and Inspections

Authorized representatives of Enterin, Inc., a regulatory authority, an Independent Ethics Committee or an Institutional Review Board may visit the site to perform audits or inspections, including source data verification. The purpose of 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 guidelines of the International Conference on

Harmonization, and any applicable regulatory requirements. The investigator should contact Enterin, Inc. immediately if contacted by a regulatory agency about an inspection.

12.5 Ethics Committee Review

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

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

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

12.6 Standards

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

12.7 Confidentiality

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

However, information obtained from individual subject's participation in the study may be disclosed with his/her consent to the health care providers for the purpose of obtaining appropriate medical care. The subject's medical records/charts, tests with his/her name on them may be made

available to the appropriate contract research organization (CRO), the sponsor, its potential eventual partners, and any other regulatory authorities. This is for the purpose of verifying information obtained for this study. Confidentiality will be maintained throughout the study within the limits of the law.

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

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

12.8 Protocol Adherence

The pharmacist or other designated individual will maintain records of study treatment delivered to the study site; the inventory at the site; the distribution to and use by each patient; 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 patients.

At each visit after initiation of treatment, site staff will record compliance of patients with their assigned regimen. Patients will be instructed to bring their diaries and cartons containing unused/partially used/empty vials back for inspection at each study visit. Patients 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 patients were provided with the correct study drug and will reconcile the products received from the drug dispensing center. Investigational product will not be returned to 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 outpatient evaluation period (visit to visit). Discontinuation for noncompliance is at the Investigator's discretion and is to be noted on the case report form (CRF).

12.9 Amendments to the Protocol

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

12.10 Protocol Deviations

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

12.11 Study Termination

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

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

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

12.12 Data handling and record keeping

12.13 Inspection of Records

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

12.14 Data Management

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

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

12.15 Data Capture and Management

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

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

The Investigator, or designated representative, should complete the eCRF as soon as possible after information is collected, preferably on the same day that a subject is seen for an examination, treatment, or any other study procedure. The information collected from the daily sleep and stool diaries will be entered by the sponsor or a designated representative 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.

12.16 Liability and Insurance

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

12.17 Retention of Records

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

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

12.18 Data Quality Assurance

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

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

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

13 USE OF INFORMATION

Both the use of data and the publication policy are detailed within the clinical study agreement. Intellectual property rights (and related matters) generated by the Investigator and others performing the clinical study will be patient 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, patient to the terms of any such agreement. In order to facilitate such ownership, Investigators will be required to assign all such inventions directly to Enterin, Inc. or its designee, as will be set forth in the clinical study agreement.

14 INVESTIGATOR AGREEMENT

I have read the foregoing protocol "A Multicenter, Single-dose, Multiple-dose, Randomized, Double-blind, Placebo-controlled study to evaluate Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of orally administered ENT-01 for Constipation in Parkinson's disease" and agree to conduct the study as described therein.

[name]

Investigator's Name

[dd/mm/yyyy]

Investigator's Signature | Date

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APPENDIX 1: CLINICAL LABORATORY DETERMINATION

Hematology	**Serum chemistry**	**Urine analysis (dipstick)**
Full and differential blood count Hematocrit (Hct) Hemoglobin (Hb) Mean corpuscular hemoglobin (MCH) Mean corpuscular hemoglobin concentration (MCHC) Mean corpuscular volume (MCV) Platelet count Red blood cell (RBC) count White blood cell (WBC) count with differential	Albumin Alanine aminotransferase (ALT) Alkaline phosphatase (ALP) Aspartate aminotransferase (AST) Blood Urea Nitrogen (BUN) or Urea Carbon dioxide (CO_2) Creatinine Creatine kinase and subtypes Electrolytes (Na, K, Cl, Ca, P) Gamma--glutamyl transpeptidase (GGT) Glucose Lactate dehydrogenase (LDH)? Total bilirubin Direct bilirubin Total cholesterol Triglycerides	Appearance pH Protein Glucose Ketone bodies Indicators of blood and WBCs Specific gravity Urine human chorionic gonadotropin (HCG) (pre-menopausal females only) Urobilinogen
Coagulation		
Prothrombin time (PT) Activated partial thromboplastin time (PTT)		
Pregnancy test: A pregnancy test will be performed on all female patients of child-bearing potential at the screening visit.		

APPENDIX 2: SCHEDULES OF EVENTS

STAGE 1

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

BDI	x		X[4]	x[2]
Trail-making A and B	x		X[4]	x[2]
MMSE	x		X[4]	x[2]
Adverse events		x	X	x
PK samples			X[5]	
Schedule visit days	x			
Administer ENT Dose			x	
Record Medications	x	x	x	x
Daily phone calls to on non-visit days		x	x	x

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

[2]End of period 2

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

[4]Surveys taken at last dosing visit

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

STAGE 2

	Period 3.1 Run-In					Period 3.2 Multiple Dose		Period 4 Randomized Treatment			Period 5 Wash-Out	
	Day 1 Visit 1	Days 2-7, 9-10, 12-13 Calls	Day 8 Call	Day 11 Visit 2	Day 14 Call	Days 1-13, 15-16 Calls	Day 14 Call	Day 1 Visit 3[6]	Days 2-5 Calls	Day 6 Visit 4	Days 1-7 Calls	Day 14 Visit 5
Informed Consent	X											
Inclusion/Exclusion Criteria	X											
Demographics/Medical History	X											
Prior and Con Meds	X											
Complete Physical Exam	X											X
Height	X											
Weight	X			X				X		X		X
Vital Signs[1]	X			X				X		X		X
ECG	X							X				X
Local Labs - Chemistry, Hematology	X							X		X		X
Local Labs - Urinalysis, Serum Pregnancy	X											
Fecal Occult Blood	X							X				X
Randomization								X				
Study Drug Dispense, Accountability, or Collection				X				X		X		
Reminder to Begin Taking Study Drug the Next Morning					X							
Instructions to Discontinue Laxatives and other BM Medications	X											
Instructions to Resume Laxatives and other BM medications												X
Stool and Sleep eDiary Instruction/Review	X	X	X	X	X	X	X	X	X	X	X	X
AE Review		X	X	X	X	X	X	X	X	X	X	X
Rome IV questionnaire	X		X									
UPDRS	X							X				X
PAC-QOL and PAC-SYM	X				X		X	X		X		X
Questionnaires - BDI, NMSQ, PFS-16, UM-PDHQ, MMSE, Trail-Making A and B, RBDQ and PDSS	X							X		X		X
Sitzmarks Capsule Distributions[2]	X							X				
Reminder to Take Sitzmarks Capsules[3]		X							X			

	Period 3.1 Run-In					Period 3.2 Multiple Dose		Period 4 Randomized Treatment			Period 5 Wash-Out	
	Day 1 Visit 1	Days 2-7, 9-10, 12-13 Calls	Day 8 Call	Day 11 Visit 2	Day 14 Call	Days 1-13, 15-16 Calls	Day 14 Call	Day 1 Visit 3[6]	Days 2-5 Calls	Day 6 Visit 4	Days 1-7 Calls	Day 14 Visit 5
Reminder to Take Sitzmarks Capsules[3]		X							X			
Abdominal X-Ray				X						X		
Dispense I-Button[4]	X											
PK Draws & LFT[5]								X		X		
Phone Call		X	X		X	X	X		X		X	

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

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

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

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

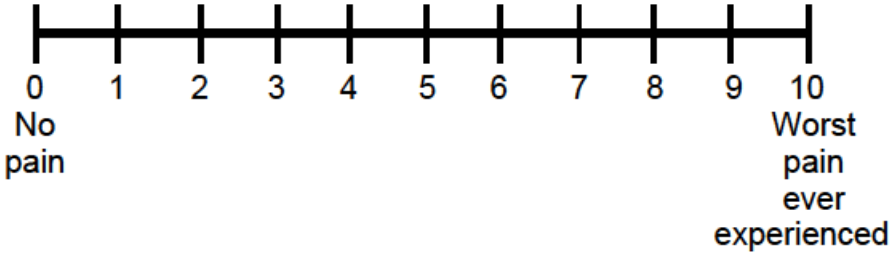
[5]PK samples are taken pre-dose and 1, 2, 4, and 8 hours post-dose. A sample for liver function tests will be taken with the 8-hour post-dose PK draw.

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

APPENDIX 3: NUMERIC RATING SCALES FOR PAIN AND SWELLING

0–10 Numeric Pain Rating Scale

On a pain scale of 0-10, where "0" is no pain and "10" is the worst pain you have ever had, please indicate the amount of pain you are currently having associated with your flare-up?



APPENDIX 4: ROME-IV CRITERIA FOR CONSTIPATION

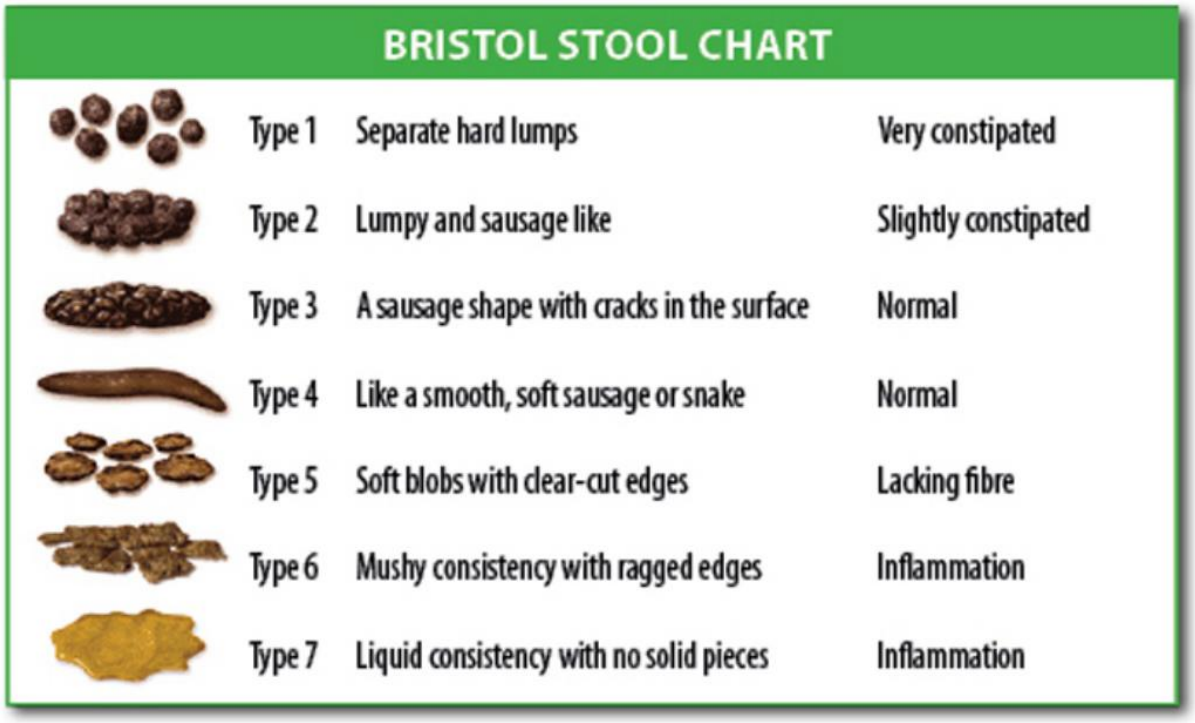
Must include two or more of the following:
Straining during at least 25% of defecations
Lumpy or hard stools in at least 25% of defecations
Sensation of incomplete evacuation for at least 25% of defecations
Sensation of incomplete evacuation for at least 25% of defecations
Sensation of anorectal obstruction/block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

APPENDIX 5: EASE OF EVACUATION SCALE

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

APPENDIX 6: BRISTOL STOOL CHART

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



APPENDIX 7: SLEEP DIARY

Participants will complete a sleep diary on a daily basis throughout the study.
The diaries will include time into bed and estimated time to sleep as well as wake time and duration during the night.

An example of the sleep diary is shown below:

Date	**Dose (mg)**	**Time to bed (hr)**	**Sleep onset (hr)**	**Morning awakening (hr)**	**Wakes at night/ duration (min)**	**Total Hours sleep**	**Leg thrashing/screaming/vivid dreams**	**I-Button on**
3/20	-	12	1.30	7.0	2 (30min each)	4.5	Yes	No
3/21	100	12	1.0	7.0	1 (60 min)	5.0	Some	Yes,
3/22	100	11	12.30	7.30	2 (60 min, 60min)	5.0	Yes	Yes,
3/23	100	12	12.30	6.30	1 (30min)	5.5	Some	Yes,
3/24	150	-	11.0	7.30	1 (30min)	8.0	No	Yes,
3/25	150	-	11.0	7.0	-	8.0	No	Yes,

APPENDIX 8: I-BUTTON

The I-Button is a small rugged self-sufficient system that measures temperature and records the results in a protected memory section. The Thermochron I-Button DS1921H (Maxim Integrated, Dallas TX) used for skin temperature measurement. The I-Button DS1921H is regulated by Federal Communications Commission (FCC) as a class B digital radiofrequency device that is an unintentional radiator (i.e., CFR 47, Part 15, Subpart B), and conforms to the national standards tested under ANSI C63.4-2001. The I-Button is composed of a semiconductor temperature sensor, a real timer, a memory and a lithium battery, all enclosed in a 16-mm diameter by 6-mm thick cylinder. Manufacturing specifications include: temperature range from +15 to +46 °C, and 1 °C of accuracy with a precision of 0.125 °C. The memory (NV RAM) allows it to record up to 2048 values taken at regular intervals of 1 to 255 min. I-Buttons will be programmed to sample every 10 min, and attached to a double-sided cotton sport wrist band using Velcro®, with the sensor face of the I-Button being placed over the inside of the wrist, on the radial artery of the dominant hand. Subjects will remove and replace the data logger when necessary (i.e., to have a bath or shower). After the period of monitoring, the information stored in the I-Button will be transferred through an adapter (DS1402D-DR8, IDC, Spain) to a personal computer using I-Button Viewer v. 3.22© 1992–2005 Dallas Semiconductor MAXIM software provided by the manufacturer. The value of skin temperature assessment in sleep research is that the endogenous skin warming resulting from increased skin blood flow is functionally linked to sleep propensity (Sarabia et al., 2008; van Marken Lichtenbelt et al., 2006).

From the collected data we will calculate the mesor, amplitude,acrophase (time of peak temperature), Rayleigh test (an index of interdaily stability), mean waveforms (averaged per subject, and then for each experimental group) using an analysis package for temporal series. Briefly, interdaily phase stability will be calculated as follows: firstly, daily acrophases for subjects at baseline and following treatment will be obtained using least-squares data fitting to a cosine function with a period of 24 h. The acrophase distribution within a 24-h period will be assessed using a Rayleigh test (CircStat: Matlab toolbox for circular statistical package). This test provides an r vector with its origin at the center of a circumference of radius one. The r vector length (between 0 and 1) is proportional to the degree of phase homogeneity during the period analyzed, and can be considered to be a measure of the rhythm's phase stability during successive days.

Statistical differences between baseline and treatment values will be analyzed by two-way repeated measures ANOVA(Sarabia et al., 2008).

APPENDIX 9: NON-MOTOR SYMPTOMS QUESTIONNAIRE (NMSQ)

Have you experienced any of the following in the last month?

		Yes	No
1	Dribbling of saliva during the daytime.	☐	☐
2	Loss or change in your ability to taste or smell.	☐	☐
3	Difficulty swallowing food or drink or problems with choking.	☐	☐
4	Vomiting or feelings of sickness (nausea).	☐	☐
5	Constipation (less than three bowel movements a week) or having to strain to pass a stool.	☐	☐
6	Bowel (faecal) incontinence.	☐	☐
7	Feeling that your bowel emptying is incomplete after having been to the toilet.	☐	☐
8	A sense of urgency to pass urine makes you rush to the toilet.	☐	☐
9	Getting up regularly at night to pass urine.	☐	☐
10	Unexplained pains (not due to known conditions such as arthritis).	☐	☐
11	Unexplained change in weight (not due to change in diet).	☐	☐
12	Problems remembering things that have happened recently or forgetting to do things.	☐	☐
13	Loss of interest in what is happening around you or in doing things.	☐	☐
14	Seeing or hearing things that you know or are told are not there.	☐	☐
15	Difficulty concentrating or staying focussed.	☐	☐
16	Feeling sad, 'low' or 'blue'.	☐	☐
17	Feeling anxious, frightened or panicky.	☐	☐
18	Feeling less interested in sex or more interested in sex.	☐	☐
19	Finding it difficult to have sex when you try.	☐	☐
20	Feeling light-headed, dizzy or weak standing from sitting or lying.	☐	☐
21	Falling.	☐	☐
22	Finding it difficult to stay awake during activities such as working, driving or eating.	☐	☐
23	Difficulty getting to sleep at night or staying asleep at night.	☐	☐
24	Intense, vivid or frightening dreams.	☐	☐
25	Talking or moving about in your sleep, as if you are 'acting out' a dream.	☐	☐
26	Unpleasant sensations in your legs at night or while resting, and a feeling that you need to move.	☐	☐
27	Swelling of the legs.	☐	☐
28	Excessive sweating.	☐	☐
29	Double vision.	☐	☐
30	Believing things are happening to you that other people say are not.	☐	☐

APPENDIX 10: BECK DEPRESSION INVENTORY (BDI)

1.

0 I do not feel sad.

1 I feel sad

2 I am sad all the time and I can't snap out of it.

3 I am so sad and unhappy that I can't stand it.

2.

0 I am not particularly discouraged about the future.

1 I feel discouraged about the future.

2 I feel I have nothing to look forward to.

3 I feel the future is hopeless and that things cannot improve.

3.

0 I do not feel like a failure.

1 I feel I have failed more than the average person.

2 As I look back on my life, all I can see is a lot of failures.

3 I feel I am a complete failure as a person.

4.

0 I get as much satisfaction out of things as I used to.

1 I don't enjoy things the way I used to.

2 I don't get real satisfaction out of anything anymore.

3 I am dissatisfied or bored with everything.

5.

0 I don't feel particularly guilty

1 I feel guilty a good part of the time.

2 I feel quite guilty most of the time.

3 I feel guilty all of the time.

6.

0 I don't feel I am being punished.

1 I feel I may be punished.

2 I expect to be punished.

3 I feel I am being punished.

7.

0 I don't feel disappointed in myself.

1 I am disappointed in myself.

2 I am disgusted with myself.

3 I hate myself.

8.

0 I don't feel I am any worse than anybody else.

1 I am critical of myself for my weaknesses or mistakes.

2 I blame myself all the time for my faults.

3 I blame myself for everything bad that happens.

9.

0 I don't have any thoughts of killing myself.

1 I have thoughts of killing myself, but I would not carry them out.

2 I would like to kill myself.

3 I would kill myself if I had the chance.

10.

0 I don't cry any more than usual.

1 I cry more now than I used to.

2 I cry all the time now.

3 I used to be able to cry, but now I can't cry even though I want to.

11.

0 I am no more irritated by things than I ever was.

1 I am slightly more irritated now than usual.

2 I am quite annoyed or irritated a good deal of the time.

3 I feel irritated all the time.

12.

0 I have not lost interest in other people.

1 I am less interested in other people than I used to be.

2 I have lost most of my interest in other people.

3 I have lost all of my interest in other people.

13.

0 I make decisions about as well as I ever could.

1 I put off making decisions more than I used to.

2 I have greater difficulty in making decisions more than I used to.

3 I can't make decisions at all anymore.

14.

0 I don't feel that I look any worse than I used to.

1 I am worried that I am looking old or unattractive.

2 I feel there are permanent changes in my appearance that make me look unattractive

3 I believe that I look ugly.

15.

0 I can work about as well as before.

1 It takes an extra effort to get started at doing something.

2 I have to push myself very hard to do anything.

3 I can't do any work at all.

16.

0 I can sleep as well as usual.

1 I don't sleep as well as I used to.

2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.

3 I wake up several hours earlier than I used to and cannot get back to sleep.

17.

0 I don't get more tired than usual.

1 I get tired more easily than I used to.

2 I get tired from doing almost anything.

3 I am too tired to do anything.

18.

0 My appetite is no worse than usual.

1 My appetite is not as good as it used to be.

2 My appetite is much worse now.

3 I have no appetite at all anymore.

19.

0 I haven't lost much weight, if any, lately.

1 I have lost more than five pounds.

2 I have lost more than ten pounds.

3 I have lost more than fifteen pounds.

20.

0 I am no more worried about my health than usual.

1 I am worried about physical problems like aches, pains, upset stomach, or constipation.

2 I am very worried about physical problems and it's hard to think of much else.

3 I am so worried about my physical problems that I cannot think of anything else.

21.

0 I have not noticed any recent change in my interest in sex.

1 I am less interested in sex than I used to be.

2 I have almost no interest in sex.

3 I have lost interest in sex completely.

INTERPRETING THE BECK DEPRESSION INVENTORY Now that you have completed the questionnaire, add up the score for each of the twenty-one questions by counting the number to the right of each question you marked. The highest possible total for the whole test would be sixty-three. This would mean you circled number three on all twenty-one questions. Since the lowest possible score for each question is zero, the lowest possible score for the test would be zero. This would mean you circles zero on each question. You can evaluate your depression according to the Table below. Total Score_____

Levels of Depression 1-10_____These ups and downs are considered normal

11-16_____ Mild mood disturbance

17-20_____Borderline clinical depression

21-30_____Moderate depression

31-40_____Severe depression

over 40_____Extreme depression

APPENDIX 11: UNIFIED PARKINSON'S DISEASE RATING SCALE

Part I: Non-Motor Aspects of Experiences of Daily Living (nM-EDL)

Overview: This portion of the scale assesses the non-motor impact of Parkinson's disease (PD) on patients' experiences of daily living. There are 13 questions. Part 1A is administered by the rater (six questions) and focuses on complex behaviors. Part 1B is a component of the self-administered Patient Questionnaire that covers seven questions on non-motor experiences of daily living.

Part 1A:
In administering Part IA, the examiner should use the following guidelines:

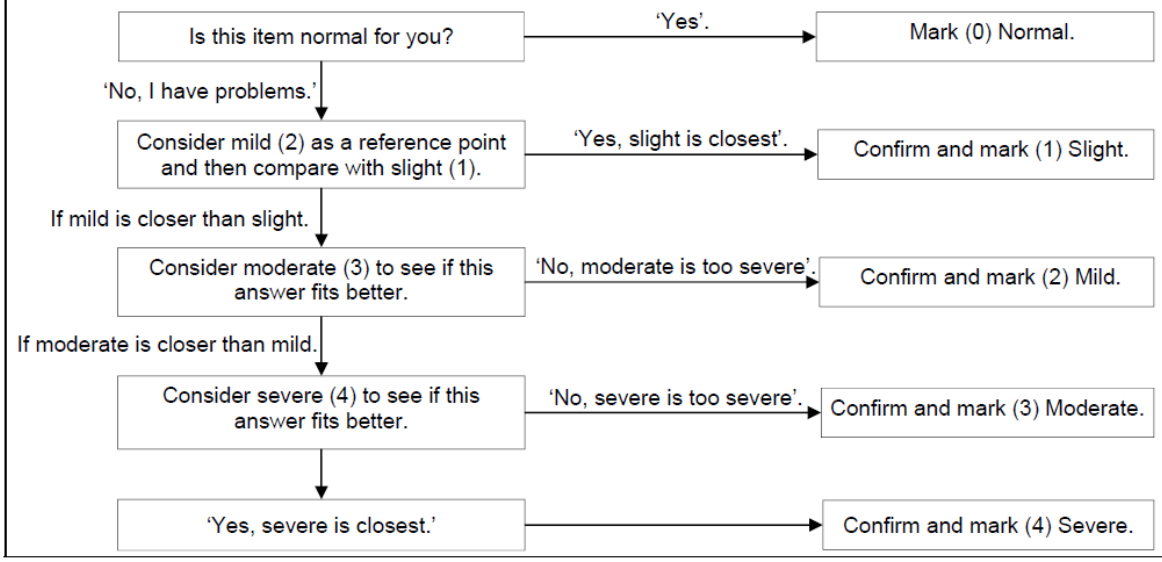
1. Mark at the top of the form the primary data source as patient, caregiver, or patient and caregiver in equal proportion.
2. The response to each item should refer to a period encompassing the prior week including the day on which the information is collected.
3. All items must have an integer rating (no half points, no missing scores). In the event that an item does not apply or cannot be rated (e.g., amputee who cannot walk), the item is marked UR for Unable to Rate.
4. The answers should reflect the usual level of function and words such as "usually", "generally", "most of the time" can be used with patients.
5. Each question has a text for you to read (Instructions to patients/caregiver). After that statement, you can elaborate and probe based on the target symptoms outlined in the Instructions to examiner. You should NOT READ the RATING OPTIONS to the patient/caregiver, because these are written in medical terminology. From the interview and probing, you will use your medical judgment to arrive at the best response.
6. Patients may have co-morbidities and other medical conditions that can affect their function. You and the patient must rate the problem as it exists and do not attempt to separate elements due to Parkinson's disease from other conditions.

EXAMPLE OF NAVIGATING THROUGH THE RESPONSE OPTIONS FOR PART 1A

Suggested strategies for obtaining the most accurate answer:
After reading the instructions to the patient, you will need to probe the entire domain under discussion to determine Normal vs. problematic: If your questions do not identify any problem in this domain, record 0 and move on to the next question.

If your questions identify a problem in this domain, you should work next with a reference anchor at the mid-range (option 2 or Mild) to find out if the patient functions at this level, better or worse. You will not be reading the choices of responses to the patient as the responses use clinical terminology. You will be asking enough probing questions to determine the response that should be coded.

Work up and down the options with the patient to identify the most accurate response, giving a final check by excluding the options above and below the selected response.



MDS UPDRS
Part I: Non-Motor Aspects of Experiences of Daily Living (nM-EDL)

Part 1A: Complex behaviors: [completed by rater]

Primary source of information:

☐ Patient ☐ Caregiver ☐ Patient and Caregiver in Equal Proportion

To be read to the patient: I am going to ask you six questions about behaviors that you may or may not experience. Some questions concern common problems and some concern uncommon ones. If you have a problem in one of the areas, please choose the best response that describes how you have felt MOST OF THE TIME during the PAST WEEK. If you are not bothered by a problem, you can simply respond NO. I am trying to be thorough, so I may ask questions that have nothing to do with you.

1.1 COGNITIVE IMPAIRMENT	SCORE
Instructions to examiner: Consider all types of altered level of cognitive function including cognitive slowing, impaired reasoning, memory loss, deficits in attention and orientation. Rate their impact on activities of daily living as perceived by the patient and/or caregiver. *Instructions to patients [and caregiver]: Over the past week have you had problems remembering things, following conversations, paying attention, thinking clearly, or finding your way around the house or in town? [If yes, examiner asks patient or caregiver to elaborate and probes for information]* 0: Normal: No cognitive impairment. 1: Slight: Impairment appreciated by patient or caregiver with no concrete interference with the patient's ability to carry out normal activities and social interactions. 2: Mild: Clinically evident cognitive dysfunction, but only minimal interference with the patient's ability to carry out normal activities and social interactions. 3: Moderate: Cognitive deficits interfere with but do not preclude the patient's ability to carry out normal activities and social interactions. 4: Severe: Cognitive dysfunction precludes the patient's ability to carry out normal activities and social interactions.	☐

	SCORE
1.2 HALLUCINATIONS AND PSYCHOSIS Instructions to examiner: Consider both illusions (misinterpretations of real stimuli) and hallucinations (spontaneous false sensations). Consider all major sensory domains (visual, auditory, tactile, olfactory and gustatory). Determine presence of unformed (for example sense of presence or fleeting false impressions) as well as formed (fully developed and detailed) sensations. Rate the patients insight into hallucinations and identify delusions and psychotic thinking. *Instructions to patients [and caregiver]: Over the past week have you seen, heard, smelled or felt things that were not really there?* [If yes, examiner asks patient or caregiver to elaborate and probes for information] 0: Normal: No hallucinations or psychotic behaviour. 1: Slight: Illusions or non-formed hallucinations, but patient recognizes them without loss of insight. 2: Mild: Formed hallucinations independent of environmental stimuli. No loss of insight. 3: Moderate: Formed hallucinations with loss of insight. 4: Severe: Patient has delusions or paranoia.	☐
1.3 DEPRESSED MOOD Instructions to examiner: Consider low mood, sadness, hopelessness, feelings of emptiness or loss of enjoyment. Determine their presence and duration over the past week and rate their interference with the patient's ability to carry out daily routines and engage in social interactions. *Instruction to the patient (and caregiver): Over the past week have you felt low, sad, hopeless or unable to enjoy things? If yes, was this feeling for longer than one day at a time? Did it make it difficult for you carry out your usual activities or to be with people?* If yes, examiner asks patient or caregiver to elaborate and probes for information] 0: Normal: No depressed mood. 1: Slight: Episodes of depressed mood that are not sustained for more than one day at a time. No interference with patient's ability to carry out normal activities and social interactions. 2: Mild: Depressed mood that is sustained over days, but without interference with normal activities and social interactions. 3: Moderate: Depressed mood that interferes with, but does not preclude, the patient's ability to carry out normal activities and social interactions. 4: Severe: Depressed mood precludes patient's ability to carry out normal activities and social interactions.	☐

	SCORE
1.4 ANXIOUS MOOD Instructions to examiner: Determine nervous, tense, worried or anxious feelings (including panic attacks) over the past week and rate their duration and interference with the patient's ability to carry out daily routines and engage in social interactions. *Instructions to patients [and caregiver]: Over the past week have you felt nervous, worried or tense? If yes, was this feeling for longer than one day at a time? Did it make it difficult for you to follow your usual activities or to be with other people?* [If yes, examiner asks patient or caregiver to elaborate and probes for information.] 0: Normal: No anxious feelings. 1: Slight: Anxious feelings present but not sustained for more than one day at a time. No interference with patient's ability to carry out normal activities and social interactions. 2: Mild: Anxious feelings are sustained over more than one day at a time, but without interference with patient's ability to carry out normal activities and social interactions. 3: Moderate: Anxious feelings interfere with, but do not preclude, the patient's ability to carry out normal activities and social interactions. 4: Severe: Anxious feelings preclude patient's ability to carry out normal activities and social interactions.	☐
1.5 APATHY Instructions to examiner: Consider level of spontaneous activity, assertiveness, motivation and initiative and rate the impact of reduced levels on performance of daily routines and social interactions. Here the examiner should attempt to distinguish between apathy and similar symptoms that are best explained by depression. *Instructions to patients (and caregiver): Over the past week, have you felt indifferent to doing activities or being with people?* If yes, examiner asks patient or caregiver to elaborate and probes for information.] 0: Normal: No apathy. 1: Slight: Apathy appreciated by patient and/or caregiver, but no interference with daily activities and social interactions. 2: Mild: Apathy interferes with isolated activities and social interactions. 3: Moderate: Apathy interferes with most activities and social interactions. 4: Severe: Passive and withdrawn, complete loss of initiative.	☐

1.6 FEATURES OF DOPAMINE DYSREGULATION SYNDROME	SCORE
Instructions to examiner: Consider involvement in a variety of activities including atypical or excessive gambling (e.g. casinos or lottery tickets), atypical or excessive sexual drive or interests (e.g., unusual interest in pornography, masturbation, sexual demands on partner), other repetitive activities (e.g. hobbies, dismantling objects, sorting or organizing), or taking extra non-prescribed medication for non-physical reasons (i.e., addictive behavior). Rate the impact of such abnormal activities/behaviors on the patient's personal life and on his family and social relations (including need to borrow money or other financial difficulties like withdrawal of credit cards, major family conflicts, lost time from work, or missed meals or sleep because of the activity). *Instructions to patients [and caregiver]: Over the past week, have you had unusually strong urges that are hard to control? Do you feel driven to do or think about something and find it hard to stop?* [Give patient examples such as gambling, cleaning, using the computer, taking extra medicine, obsessing about food or sex, all depending on the patients. 0: Normal: No problems present. 1: Slight: Problems are present but usually do not cause any difficulties for the patient or family/caregiver. 2: Mild: Problems are present and usually cause a few difficulties in the patient's personal and family life. 3: Moderate: Problems are present and usually cause a lot of difficulties in the patient's personal and family life. 4: Severe: Problems are present and preclude the patient's ability to carry out normal activities or social interactions or to maintain previous standards in personal and family life.	☐

The remaining questions in Part I (Non-motor Experiences of Daily Living) [Sleep, Daytime Sleepiness, Pain and Other Sensation, Urinary Problems, Constipation Problems, Lightheadedness on Standing, and Fatigue] are in the **Patient Questionnaire** along with all questions in Part II [Motor Experiences of Daily Living].

Patient Questionnaire:

Instructions:

This questionnaire will ask you about your experiences of daily living.

There are 20 questions. We are trying to be thorough, and some of these questions may therefore not apply to you now or ever. If you do not have the problem, simply mark 0 for NO.

Please read each one carefully and read all answers before selecting the one that best applies to you.

We are interested in your average or usual function over the past week including today. Some patients can do things better at one time of the day than at others. However, only one answer is allowed for each question, so please mark the answer that best describes what you can do most of the time.

You may have other medical conditions besides Parkinson's disease. Do not worry about separating Parkinson's disease from other conditions. Just answer the question with your best response.

Use only 0, 1, 2, 3, 4 for answers, nothing else. Do not leave any blanks.

Your doctor or nurse can review the questions with you, but this questionnaire is for patients to complete, either alone or with their caregivers.

Who is filling out this questionnaire (check the best answer):

☐ Patient ☐ Caregiver ☐ Patient and Caregiver in Equal Proportion

Part I: Non-Motor Aspects of Experiences of Daily Living (nM-EDL)	
1.7 SLEEP PROBLEMS Over the past week, have you had trouble going to sleep at night or staying asleep through the night? Consider how rested you felt after waking up in the morning. 0: Normal: No problems. 1: Slight: Sleep problems are present but usually do not cause trouble getting a full night of sleep. 2: Mild: Sleep problems usually cause some difficulties getting a full night of sleep. 3: Moderate: Sleep problems cause a lot of difficulties getting a full night of sleep, but I still usually sleep for more than half the night. 4: Severe: I usually do not sleep for most of the night.	SCORE ☐
1.8 DAYTIME SLEEPINESS Over the past week, have you had trouble staying awake during the daytime? 0: Normal: No daytime sleepiness. 1: Slight: Daytime sleepiness occurs but I can resist and I stay awake. 2: Mild: Sometimes I fall asleep when alone and relaxing. For example, while reading or watching TV. 3: Moderate: I sometimes fall asleep when I should not. For example, while eating or talking with other people. 4: Severe: I often fall asleep when I should not. For example, while eating or talking with other people.	☐

	SCORE
1.9 PAIN AND OTHER SENSATIONS Over the past week, have you had uncomfortable feelings in your body like pain, aches tingling or cramps? 0: Normal: No uncomfortable feelings. 1: Slight: I have these feelings. However, I can do things and be with other people without difficulty. 2: Mild: These feelings cause some problems when I do things or am with other people. 3: Moderate: These feelings cause a lot of problems, but they do not stop me from doing things or being with other people. 4: Severe: These feelings stop me from doing things or being with other people.	☐
1.10 URINARY PROBLEMS Over the past week, have you had trouble with urine control? For example, an urgent need to urinate, a need to urinate too often, or urine accidents? 0: Normal: No urine control problems. 1: Slight: I need to urinate often or urgently. However, these problems do not cause difficulties with my daily activities. 2: Mild: Urine problems cause some difficulties with my daily activities. However, I do not have urine accidents. 3: Moderate: Urine problems cause a lot of difficulties with my daily activities, including urine accidents. 4: Severe: I cannot control my urine and use a protective garment or have a bladder tube.	☐

	SCORE
1.11 CONSTIPATION PROBLEMS Over the past week have you had constipation troubles that cause you difficulty moving your bowels? 0: Normal: No constipation. 1: Slight: I have been constipated. I use extra effort to move my bowels. However, this problem does not disturb my activities or my being comfortable. 2: Mild: Constipation causes me to have some troubles doing things or being comfortable. 3: Moderate: Constipation causes me to have a lot of trouble doing things or being comfortable. However, it does not stop me from doing anything. 4: Severe: I usually need physical help from someone else to empty my bowels.	☐
1.12 LIGHT HEADEDNESS ON STANDING Over the past week, have you felt faint, dizzy or foggy when you stand up after sitting or lying down? 0: Normal: No dizzy or foggy feelings. 1: Slight: Dizzy or foggy feelings occur. However, they do not cause me troubles doing things. 2: Mild: Dizzy or foggy feelings cause me to hold on to something, but I do not need to sit or lie back down. 3: Moderate: Dizzy or foggy feelings cause me to sit or lie down to avoid fainting or falling. 4: Severe: Dizzy or foggy feelings cause me to fall or faint.	☐

	SCORE
1.13 FATIGUE Over the past week, have you usually felt fatigued? This feeling is <u>not</u> part of being sleepy or sad 0: Normal: No fatigue. 1: Slight: Fatigue occurs. However it does not cause me troubles doing things or being with people. 2: Mild: Fatigue causes me some troubles doing things or being with people. 3: Moderate: Fatigue causes me a lot of troubles doing things or being with people. However, it does not stop me from doing anything. 4: Severe: Fatigue stops me from doing things or being with people.	☐
Part II: Motor Aspects of Experiences of Daily Living (M-EDL)	
2.1 SPEECH Over the past week, have you had problems with your speech? 0: Normal: Not at all (no problems). 1: Slight: My speech is soft, slurred or uneven, but it does not cause others to ask me to repeat myself. 2: Mild: My speech causes people to ask me to occasionally repeat myself, but not everyday. 3: Moderate: My speech is unclear enough that others ask me to repeat myself every day even though most of my speech is understood. 4: Severe: Most or all of my speech cannot be understood.	☐

	SCORE
2.2 SALIVA & DROOLING Over the past week, have you usually had too much saliva during when you are awake or when you sleep? 0: Normal: Not at all (no problems). 1: Slight: I have too much saliva, but do not drool. 2: Mild: I have some drooling during sleep, but none when I am awake. 3: Moderate: I have some drooling when I am awake, but I usually do not need tissues or a handkerchief. 4: Severe: I have so much drooling that I regularly need to use tissues or a handkerchief to protect my clothes.	☐
2.3 CHEWING AND SWALLOWING Over the past week, have you usually had problems swallowing pills or eating meals? Do you need your pills cut or crushed or your meals to be made soft, chopped or blended to avoid choking? 0: Normal: No problems. 1: Slight: I am aware of slowness in my chewing or increased effort at swallowing, but I do not choke or need to have my food specially prepared. 2: Mild: I need to have my pills cut or my food specially prepared because of chewing or swallowing problems, but I have not choked over the past week. 3: Moderate. I choked at least once in the past week. 4: Severe: Because of chewing and swallowing problems, I need a feeding tube.	☐

	SCORE
2.4 EATING TASKS Over the past week, have you usually had troubles handling your food and using eating utensils? For example, do you have trouble handling finger foods or using forks, knifes, spoons, chopsticks? 0: Normal: Not at all (No problems). 1: Slight: I am slow, but I do not need any help handling my food and have not had food spills while eating. 2: Mild: I am slow with my eating and have occasional food spills. I may need help with a few tasks such as cutting meat. 3: Moderate: I need help with many eating tasks but can manage some alone. 4: Severe: I need help for most or all eating tasks.	☐
2.5 DRESSING Over the past week, have you usually had problems dressing? For example, are you slow or do you need help with buttoning, using zippers, putting on or taking off your clothes or jewelry? 0: Normal: Not at all (no problems). 1: Slight: I am slow but I do not need help. 2: Mild: I am slow and need help for a few dressing tasks (buttons, bracelets). 3: Moderate: I need help for many dressing tasks. 4: Severe: I need help for most or all dressing tasks.	☐

	SCORE
2.6 HYGIENE Over the past week, have you usually been slow or do you need help with washing, bathing, shaving, brushing teeth, combing your hair or with other personal hygiene? 0: Normal: Not at all (no problems). 1: Slight: I am slow but I do not need any help. 2: Mild: I need someone else to help me with some hygiene tasks. 3: Moderate: I need help for many hygiene tasks. 4: Severe: I need help for most or all of my hygiene tasks.	☐
2.7 HANDWRITING Over the past week, have people usually had trouble reading your handwriting? 0: Normal: Not at all (no problems). 1: Slight: My writing is slow, clumsy or uneven, but all words are clear. 2: Mild: Some words are unclear and difficult to read. 3: Moderate: Many words are unclear and difficult to read. 4: Severe: Most or all words cannot be read.	☐
2.8 DOING HOBBIES AND OTHER ACTIVITIES Over the past week, have you usually had trouble doing your hobbies or other things that you like to do? 0: Normal: Not at all (no problems). 1: Slight: I am a bit slow but do these activities easily. 2: Mild: I have some difficulty doing these activities. 3: Moderate: I have major problems doing these activities, but still do most. 4: Severe: I am unable to do most or all of these activities.	☐

	SCORE
2.9 TURNING IN BED Over the past week, do you usually have trouble turning over in bed? 0: Normal: Not at all (no problems). 1: Slight: I have a bit of trouble turning, but I do not need any help. 2: Mild I have a lot of trouble turning and need occasional help from someone else. 3: Moderate: To turn over I often need help from someone else. 4: Severe: I am unable to turn over without help from someone else.	☐
2.10 TREMOR Over the past week, have you usually had shaking or tremor? 0: Normal: Not at all. I have no shaking or tremor. 1: Slight: Shaking or tremor occurs but does not cause problems with any activities. 2: Mild: Shaking or tremor causes problems with only a few activities. 3: Moderate: Shaking or tremor causes problems with many of my daily activities. 4: Severe: Shaking or tremor causes problems with most or all activities.	☐
2.11 GETTING OUT OF BED, A CAR, OR A DEEP CHAIR Over the past week, have you usually had trouble getting out of bed, a car seat, or a deep chair? 0: Normal: Not at all (no problems). 1: Slight: I am slow or awkward, but I usually can do it on my first try. 2: Mild: I need more than one try to get up or need occasional help. 3: Moderate: I sometimes need help to get up, but most times I can still do it on my own. 4: Severe: I need help most or all of the time.	☐

	SCORE
2.12 WALKING AND BALANCE Over the past week, have you usually had problems with balance and walking? 0: Normal: Not at all (no problems). 1: Slight: I am slightly slow or may drag a leg. I never use a walking aid. 2: Mild: I occasionally use a walking aid, but I do not need any help from another person. 3: Moderate: I usually use a walking aid (cane, walker) to walk safely without falling. However, I do not usually need the support of another person. 4: Severe: I usually use the support of another persons to walk safely without falling.	☐
2.13 FREEZING Over the past week, on your usual day when walking, do you suddenly stop or freeze as if your feet are stuck to the floor. 0: Normal: Not at all (no problems). 1: Slight: I briefly freeze but I can easily start walking again. I do not need help from someone else or a walking aid (cane or walker) because of freezing. 2: Mild: I freeze and have trouble starting to walk again, but I do not need someone's help or a walking aid (cane or walker) because of freezing. 3: Moderate: When I freeze I have a lot of trouble starting to walk again and, because of freezing, I sometimes need to use a walking aid or need someone else's help. 4: Severe: Because of freezing, most or all of the time, I need to use a walking aid or someone's help.	☐

This completes the questionnaire. We may have asked about problems you do not even have, and may have mentioned problems that you may never develop at all. Not all patients develop all these problems, but because they can occur, it is important to ask all the questions to every patient. Thank you for your time and attention in completing this questionnaire.

Part III: Motor Examination

Overview: This portion of the scale assesses the motor signs of PD. In administering Part III of the MDS-UPDRS the examiner should comply with the following guidelines:

At the top of the form, mark whether the patient is on medication for treating the symptoms of Parkinson's disease and, if on levodopa, the time since the last dose.

Also, if the patient is receiving medication for treating the symptoms of Parkinson's Disease, mark the patient's clinical state using the following definitions:

ON is the typical functional state when patients are receiving medication and have a good response.
OFF is the typical functional state when patients have a poor response in spite of taking medications.

The investigator should "rate what you see". Admittedly, concurrent medical problems such as stroke, paralysis, arthritis, contracture, and orthopedic problems such as hip or knee replacement and scoliosis may interfere with individual items in the motor examination. In situations where it is absolutely impossible to test (e.g., amputations, plegia, limb in a cast), use the notation "**UR**" for Unable to Rate. Otherwise, rate the performance of each task as the patient performs in the context of co-morbidities.

All items must have an integer rating (no half points, no missing ratings).

Specific instructions are provided for the testing of each item. These should be followed in all instances. The investigator demonstrates while describing tasks the patient is to perform and rates function immediately thereafter. For Global Spontaneous Movement and Rest Tremor items (3.14 and 3.17), these items have been placed purposefully at the end of the scale because clinical information pertinent to the score will be obtained throughout the entire examination.

At the end of the rating, indicate if dyskinesia (chorea or dystonia) was present at the time of the examination, and if so, whether these movements interfered with the motor examination.

3a Is the patient on medication for treating the symptoms of Parkinson's Disease? ☐ No ☐ Yes

3b If the patient is receiving medication for treating the symptoms of Parkinson's Disease, mark the patient's clinical state using the following definitions:

☐ ON: On is the typical functional state when patients are receiving medication and have a good response.

☐ OFF: Off is the typical functional state when patients have a poor response in spite of taking medications.

3c Is the patient on Levodopa ? ☐ No ☐ Yes

3.C1 If yes, minutes since last levodopa dose: _____

	SCORE
3.1 SPEECH Instructions to examiner: Listen to the patient's free-flowing speech and engage in conversation if necessary. Suggested topics: ask about the patient's work, hobbies, exercise, or how he got to the doctor's office. Evaluate volume, modulation (prosody) and clarity, including slurring, palilalia (repetition of syllables) and tachyphemia (rapid speech, running syllables together). 0: Normal: No speech problems. 1: Slight: Loss of modulation, diction or volume, but still all words easy to understand. 2: Mild: Loss of modulation, diction, or volume, with a few words unclear, but the overall sentences easy to follow. 3: Moderate: Speech is difficult to understand to the point that some, but not most, sentences are poorly understood. 4: Severe: Most speech is difficult to understand or unintelligible.	☐
3.2 FACIAL EXPRESSION Instructions to examiner: Observe the patient sitting at rest for 10 seconds, without talking and also while talking. Observe eye-blink frequency, masked facies or loss of facial expression, spontaneous smiling and parting of lips. 0: Normal: Normal facial expression. 1: Slight: Minimal masked facies manifested only by decreased frequency of blinking. 2: Mild: In addition to decreased eye-blink frequency, Masked facies present in the lower face as well, namely fewer movements around the mouth, such as less spontaneous smiling, but lips not parted. 3: Moderate: Masked facies with lips parted some of the time when the mouth is at rest. 4: Severe: Masked facies with lips parted most of the time when the mouth is at rest.	☐

	SCORE
3.3 RIGIDITY Instructions to examiner: Rigidity is judged on slow passive movement of major joints with the patient in a relaxed position and the examiner manipulating the limbs and neck. First, test without an activation maneuver. Test and rate neck and each limb separately. For arms, test the wrist and elbow joints simultaneously. For legs, test the hip and knee joints simultaneously. If no rigidity is detected, use an activation maneuver such as tapping fingers, fist opening/closing, or heel tapping in a limb not being tested. Explain to the patient to go as limp as possible as you test for rigidity. 0: Normal: No rigidity. 1: Slight: Rigidity only detected with activation maneuver. 2: Mild: Rigidity detected without the activation maneuver, but full range of motion is easily achieved. 3: Moderate: Rigidity detected without the activation maneuver; full range of motion is achieved with effort. 4: Severe: Rigidity detected without the activation maneuver and full range of motion not achieved.	☐ Neck ☐ RUE ☐ LUE ☐ RLE ☐ LLE
3.4 FINGER TAPPING Instructions to examiner: Each hand is tested separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to tap the index finger on the thumb 10 times as quickly AND as big as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude. 0: Normal: No problems. 1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the tapping movement; b) slight slowing; c) the amplitude decrements near the end of the 10 taps. 2: Mild: Any of the following: a) 3 to 5 interruptions during tapping; b) mild slowing; c) the amplitude decrements midway in the 10-tap sequence. 3: Moderate: Any of the following: a) more than 5 interruptions during tapping or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) the amplitude decrements starting after the 1st tap. 4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.	☐ R ☐ L

	SCORE
3.5 HAND MOVEMENTS Instructions to examiner: Test each hand separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to make a tight fist with the arm bent at the elbow so that the palm faces the examiner. Have the patient open the hand 10 times as fully AND as quickly as possible. If the patient fails to make a tight fist or to open the hand fully, remind him/ her to do so. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude. 0: Normal: No problem. 1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the movement; b) slight slowing; c) the amplitude decrements near the end of the task. 2: Mild: Any of the following: a) 3 to 5 interruptions during the movements; b) mild slowing; c) the amplitude decrements midway in the task. 3: Moderate: Any of the following: a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) the amplitude decrements starting after the 1st open-and-close sequence. 4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.	☐ R ☐ L
3.6 PRONATION-SUPINATION MOVEMENTS OF HANDS Instructions to examiner: Test each hand separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to extend the arm out in front of his/her body with the palms down; then to turn the palm up and down alternately 10 times as fast and as fully as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude. 0: Normal: No problems. 1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the movement; b) slight slowing; c) the amplitude decrements near the end of the sequence. 2: Mild: Any of the following: a) 3 to 5 interruptions during the movements; b) mild slowing; c) the amplitude decrements midway in the sequence. 3: Moderate: Any of the following: a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing c) the amplitude decrements starting after the 1st supination-pronation sequence. 4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.	☐ R ☐ L

	SCORE
3.7 TOE TAPPING Instructions to examiner: Have the patient sit in a straight-backed chair with arms, both feet on the floor. Test each foot separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to place the heel on the ground in a comfortable position and then tap the toes 10 times as big and as fast as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude. 0: Normal: No problem. 1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the tapping movement; b) slight slowing; c) amplitude decrements near the end of the ten taps. 2: Mild: Any of the following: a) 3 to 5 interruptions during the tapping movements; b) mild slowing; c) amplitude decrements midway in the task. 3: Moderate: Any of the following: a) more than 5 interruptions during the tapping movements or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing; c) amplitude decrements after the first tap. 4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.	☐ R ☐ L
3.8 LEG AGILITY Instructions to examiner: Have the patient sit in a straight-backed chair with arms. The patient should have both feet comfortably on the floor. Test each leg separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to place the foot on the ground in a comfortable position and then raise and stomp the foot on the ground 10 times as high and as fast as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude. 0: Normal: No problems. 1: Slight: Any of the following: a) the regular rhythm is broken with one or two interruptions or hesitations of the movement; b) slight slowing; c) amplitude decrements near the end of the task. 2: Mild: Any of the following: a) 3 to 5 interruptions during the movements; b) mild slowness; c) amplitude decrements midway in the task. 3: Moderate: Any of the following: a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement; b) moderate slowing in speed; c) amplitude decrements after the first tap. 4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.	☐ R ☐ L

	SCORE
3.9 ARISING FROM CHAIR Instructions to examiner: Have the patient sit in a straight-backed chair with arms, with both feet on the floor and sitting back in the chair (if the patient is not too short). Ask the patient to cross his/her arms across the chest and then to stand up. If the patient is not successful, repeat this attempt a maximum up to two more times. If still unsuccessful, allow the patient to move forward in the chair to arise with arms folded across the chest. Allow only one attempt in this situation. If unsuccessful, allow the patient to push off using his/her hands on the arms of the chair. Allow a maximum of three trials of pushing off. If still not successful, assist the patient to arise. After the patient stands up, observe the posture for item 3.13 0: Normal: No problems. Able to arise quickly without hesitation. 1: Slight: Arising is slower than normal; or may need more than one attempt; or may need to move forward in the chair to arise. No need to use the arms of the chair. 2: Mild: Pushes self up from arms of chair without difficulty. 3: Moderate: Needs to push off, but tends to fall back; or may have to try more than one time using arms of chair, but can get up without help. 4: Severe: Unable to arise without help.	☐
3.10 GAIT Instructions to examiner: Testing gait is best performed by having the patient walking away from and towards the examiner so that both right and left sides of the body can be easily observed simultaneously. The patient should walk at least 10 meters (30 feet), then turn around and return to the examiner. This item measures multiple behaviors: stride amplitude, stride speed, height of foot lift, heel strike during walking, turning, and arm swing, but not freezing. Assess also for "freezing of gait" (next item 3.11) while patient is walking. Observe posture for item 3.13 0: Normal: No problems. 1: Slight: Independent walking with minor gait impairment. 2: Mild: Independent walking but with substantial gait impairment. 3: Moderate: Requires an assistance device for safe walking (walking stick, walker) but not a person. 4: Severe: Cannot walk at all or only with another person's assistance.	☐

	SCORE
3.11 FREEZING OF GAIT Instructions to examiner: While assessing gait, also assess for the presence of any gait freezing episodes. Observe for start hesitation and stuttering movements especially when turning and reaching the end of the task. To the extent that safety permits, patients may NOT use sensory tricks during the assessment. 0: Normal: No freezing. 1: Slight: Freezes on starting, turning or walking through doorway with a single halt during any of these events, but then continues smoothly without freezing during straight walking. 2: Mild: Freezes on starting, turning or walking through doorway with more than one halt during any of these activities, but continues smoothly without freezing during straight walking. 3: Moderate: Freezes once during straight walking. 4: Severe: Freezes multiple times during straight walking.	☐
3.12 POSTURAL STABILITY Instructions to examiner: The test examines the response to sudden body displacement produced by a quick, forceful pull on the shoulders while the patient is standing erect with eyes open and feet comfortably apart and parallel to each other. Test retropulsion. Stand behind the patient and instruct the patient on what is about to happen. Explain that s/he is allowed to take a step backwards to avoid falling. There should be a solid wall behind the examiner, at least 1-2 meters away to allow for the observation of the number of retropulsive steps. The first pull is an instructional demonstration and is purposely milder and not rated. The second time the shoulders are pulled briskly and forcefully towards the examiner with enough force to displace the center of gravity so that patient MUST take a step backwards. The examiner needs to be ready to catch the patient, but must stand sufficiently back so as to allow enough room for the patient to take several steps to recover independently. Do not allow the patient to flex the body abnormally forward in anticipation of the pull. Observe for the number of steps backwards or falling. Up to and including two steps for recovery is considered normal, so abnormal ratings begin with three steps. If the patient fails to understand the test, the examiner can repeat the test so that the rating is based on an assessment that the examiner feels reflects the patient's limitations rather than misunderstanding or lack of preparedness. Observe standing posture for item 3.13 0: Normal: No problems: Recovers with one or two steps. 1: Slight: 3-5 steps, but subject recovers unaided. 2: Mild: More than 5 steps, but subject recovers unaided. 3: Moderate: Stands safely, but with absence of postural response; falls if not caught by examiner. 4: Severe: Very unstable, tends to lose balance spontaneously or with just a gentle pull on the shoulders.	☐

	SCORE
3.13 POSTURE Instructions to examiner: Posture is assessed with the patient standing erect after arising from a chair, during walking , and while being tested for postural reflexes. If you notice poor posture, tell the patient to stand up straight and see if the posture improves (see option 2 below). Rate the worst posture seen in these three observation points. Observe for flexion and side-to-side leaning. 0: Normal: No problems. 1: Slight: Not quite erect, but posture could be normal for older person. 2: Mild: Definite flexion, scoliosis or leaning to one side, but patient can correct posture to normal posture when asked to do so. 3: Moderate: Stooped posture, scoliosis or leaning to one side that cannot be corrected volitionally to a normal posture by the patient. 4: Severe: Flexion, scoliosis or leaning with extreme abnormality of posture.	☐
3.14 GLOBAL SPONTANEITY OF MOVEMENT (BODY BRADYKINESIA) Instructions to examiner: This global rating combines all observations on slowness, hesitancy, and small amplitude and poverty of movement in general, including a reduction of gesturing and of crossing the legs. This assessment is based on the examiner's global impression after observing for spontaneous gestures while sitting, and the nature of arising and walking. 0: Normal: No problems. 1: Slight: Slight global slowness and poverty of spontaneous movements. 2: Mild: Mild global slowness and poverty of spontaneous movements. 3: Moderate: Moderate global slowness and poverty of spontaneous movements. 4: Severe: Severe global slowness and poverty of spontaneous movements.	☐
3.15 POSTURAL TREMOR OF THE HANDS Instructions to examiner: All tremor, including re-emergent rest tremor, that is present in this posture is to be included in this rating. Rate each hand separately. Rate the highest amplitude seen. Instruct the patient to stretch the arms out in front of the body with palms down. The wrist should be straight and the fingers comfortably separated so that they do not touch each other. Observe this posture for 10 seconds. 0: Normal: No tremor. 1: Slight: Tremor is present but less than 1 cm in amplitude. 2: Mild: Tremor is at least 1 but less than 3 cm in amplitude. 3: Moderate: Tremor is at least 3 but less than 10 cm in amplitude. 4: Severe: Tremor is at least 10 cm in amplitude.	☐ R ☐ L

	SCORE
3.16 KINETIC TREMOR OF THE HANDS Instructions to examiner: This is tested by the finger-to-nose maneuver. With the arm starting from the outstretched position, have the patient perform at least three finger-to-nose maneuvers with each hand reaching as far as possible to touch the examiner's finger. The finger-to-nose maneuver should be performed slowly enough not to hide any tremor that could occur with very fast arm movements. Repeat with the other hand, rating each hand separately. The tremor can be present throughout the movement or as the tremor reaches either target (nose or finger). Rate the highest amplitude seen. 0: Normal: No tremor. 1: Slight: Tremor is present but less than 1 cm in amplitude. 2: Mild: Tremor is at least 1 but less than 3 cm in amplitude. 3: Moderate: Tremor is at least 3 but less than 10 cm in amplitude. 4: Severe: Tremor is at least 10 cm in amplitude.	☐ R ☐ L
3.17 REST TREMOR AMPLITUDE Instructions to examiner: This and the next item have been placed purposefully at the end of the examination to allow the rater to gather observations on rest tremor that may appear at any time during the exam, including when quietly sitting, during walking and during activities when some body parts are moving but others are at rest. Score the maximum amplitude that is seen at any time as the final score. Rate only the amplitude and not the persistence or the intermittency of the tremor. As part of this rating, the patient should sit quietly in a chair with the hands placed on the arms of the chair (not in the lap) and the feet comfortably supported on the floor for 10 seconds with no other directives. Rest tremor is assessed separately for all four limbs and also for the lip/jaw. Rate only the maximum amplitude that is seen at any time as the final rating. Extremity ratings 0: Normal: No tremor. 1: Slight.: < 1 cm in maximal amplitude. 2: Mild: > 1 cm but < 3 cm in maximal amplitude. 3: Moderate: 3 - 10 cm in maximal amplitude. 4: Severe: > 10 cm in maximal amplitude. Lip/Jaw ratings 0: Normal: No tremor. 1: Slight: < 1 cm in maximal amplitude. 2: Mild: > 1 cm but < 2 cm in maximal amplitude. 3: Moderate: > 2 cm but < 3 cm in maximal amplitude. 4: Severe: > 3 cm in maximal amplitude.	☐ RUE ☐ LUE ☐ RLE ☐ LLE ☐ Lip/Jaw

	SCORE
3.18 CONSTANCY OF REST TREMOR Instructions to examiner: This item receives one rating for all rest tremor and focuses on the constancy of rest tremor during the examination period when different body parts are variously at rest. It is rated purposefully at the end of the examination so that several minutes of information can be coalesced into the rating. 0: Normal: No tremor. 1: Slight: Tremor at rest is present < 25% of the entire examination period. 2: Mild: Tremor at rest is present 26-50% of the entire examination period. 3: Moderate: Tremor at rest is present 51-75% of the entire examination period. 4: Severe: Tremor at rest is present > 75% of the entire examination period.	☐

DYSKINESIA IMPACT ON PART III RATINGS

A. Were dyskinesias (chorea or dystonia) present during examination? ☐ No ☐ Yes

B. If yes, did these movements interfere with your ratings? ☐ No ☐ Yes

HOEHN AND YAHR STAGE 0: Asymptomatic. 1: Unilateral involvement only. 2: Bilateral involvement without impairment of balance. 3: Mile to moderate involvement; some postural instability but physically independent; needs assistance to recover from pull test. 4: Severe disability; still able to walk or stand unassisted. 5: Wheelchair bound or bedridden unless aided.	☐

Part IV: Motor Complications

Overview and Instructions: In this section, the rater uses historical and objective information to assess two motor complications, dyskinesias and motor fluctuations that include OFF-state dystonia. Use all information from patient, caregiver, and the examination to answer the six questions that summarize function over the past week including today. As in the other sections, rate using only integers (no half points allowed) and leave no missing ratings. If the item cannot be rated, place UR for Unable to Rate. You will need to choose some answers based on percentages, and therefore you will need to establish how many hours generally are awake hours and use this figure as the denominator for "OFF" time and Dyskinesias. For "OFF dystonia", the total "Off" time will be the denominator. Operational definitions for examiner's use.

Dyskinesias: Involuntary random movements
Words that patients often recognize for dyskinesias include "irregular jerking", "wiggling", "twitching". It is essential to stress to the patient the difference between dyskinesias and tremor, a common error when patients are assessing dyskinesias.

Dystonia: contorted posture, often with a twisting component:
Words that patients often recognize for dystonia include "spasms", "cramps", "posture".

Motor fluctuation: Variable response to medication:
Words that patients often recognize for motor fluctuation include "wearing out", "wearing off", "roller-coaster effect", "on-off", "uneven medication effects".

OFF: Typical functional state when patients have a poor response in spite of taking mediation or the typical functional response when patients are on NO treatment for parkinsonism. Words that patients often recognize include "low time", "bad time", "shaking time", "slow time", "time when my medications don't work."

ON: Typical functional state when patients are receiving medication and have a good response:
Words that patients often recognize include "good time", "walking time", "time when my medications work."

A . DYSKINESIAS [exclusive of OFF-state dystonia]

SCORE

4.1 TIME SPENT WITH DYSKINESIAS

Instructions to examiner: Determine the hours in the usual waking day and then the hours of dyskinesias. Calculate the percentage. If the patient has dyskinesias in the office, you can point them out as a reference to ensure that patients and caregivers understand what they are rating. You may also use your own acting skills to enact the dyskinetic movements you have seen in the patient before or show them dyskinetic movements typical of other patients. Exclude from this question early morning and nighttime painful dystonia.

Instructions to patient [and caregiver]. Over the past week, how many hours do you usually sleep on a daily basis, including nighttime sleep and daytime napping? Alright, if you sleep ___ hrs, you are awake ____ hrs. Out of those awake hours, how many hours in total do you have wiggling, twitching or jerking movements? Do not count the times when you have tremor, which is a regular back and forth shaking or times when you have painful foot cramps or spasms in the early morning or at nighttime. I will ask about those later. Concentrate only on these types of wiggling, jerking and irregular movements. Add up all the time during the waking day when these usually occur. How many hours ____ (use this number for your calculation).

0: Normal:	No dyskinesias.	
1: Slight:	≤ 25% of waking day.	
2: Mild:	26 - 50% of waking day.	
3: Moderate:	51 - 75% of waking day.	
4: Severe:	> 75% of waking day.	

1. Total Hours Awake: _____
2. Total Hours with Dyskinesia: _____
3. % Dyskinesia = ((2/1)*100): _____

	SCORE
4.2 FUNCTIONAL IMPACT OF DYSKINESIAS Instructions to examiner: Determine the degree to which dyskinesias impact on the patient's daily function in terms of activities and social interactions. Use the patient's and caregiver's response to your question and your own observations during the office visit to arrive at the best answer. *Instructions to patient [and caregiver]: Over the past week, did you usually have trouble doing things or being with people when these jerking movements occurred? Did they stop you from doing things or from being with people?* 0: Normal: No dyskinesias or no impact by dyskinesias on activities or social interactions. 1: Slight: Dyskinesias impact on a few activities, but the patient usually performs all activities and participates in all social interactions during dyskinetic periods. 2: Mild: Dyskinesias impact on many activities, but the patient usually performs all activities and participates in all social interactions during dyskinetic periods. 3: Moderate: Dyskinesias impact on activities to the point that the patient usually does not perform some activities or does not usually participate in some social activities during dyskinetic episodes. 4: Severe: Dyskinesias impact on function to the point that the patient usually does not perform most activities or participate in most social interactions during dyskinetic episodes.	☐
B . MOTOR FLUCTUATIONS	
4.3 TIME SPENT IN THE OFF STATE Instructions to examiner: Use the number of waking hours derived from 4.1 and determine the hours spent in the "OFF" state. Calculate the percentage. If the patient has an OFF period in the office, you can point to this state as a reference. You may also use your knowledge of the patient to describe a typical OFF period. Additionally you may use your own acting skills to enact an OFF period you have seen in the patient before or show them OFF function typical of other patients. Mark down the typical number of OFF hours, because you will need this number for completing 4.6 *Instructions to patient [and caregiver]: Some patients with Parkinson's disease have a good effect from their medications throughout their awake hours and we call that "ON" time. Other patients take their medications but still have some hours of low time, bad time, slow time or shaking time. Doctors call these low periods "OFF" time. Over the past week, you told me before that you are generally awake ____ hrs each day. Out of these awake hours, how many hours in total do you usually have this type of low level or OFF function ____ (Use this number for your calculations).* 0: Normal: No OFF time. 1: Slight: ≤ 25% of waking day. 2: Mild: 26 - 50% of waking day. 3: Moderate: 51 - 75% of waking day. 4: Severe: > 75% of waking day. 1. Total Hours Awake: _____ 2. Total Hours OFF: _____ 3. % OFF = ((2/1)*100): _____	☐

	SCORE
4.4 FUNCTIONAL IMPACT OF FLUCTUATIONS Instructions to examiner: Determine the degree to which motor fluctuations impact on the patient's daily function in terms of activities and social interactions. This question concentrates on the difference between the ON state and the OFF state. If the patient has no OFF time, the rating must be 0, but if patients have very mild fluctuations, it is still possible to be rated 0 on this item if no impact on activities occurs. Use the patient's and caregiver's response to your question and your own observations during the office visit to arrive at the best answer. *Instructions to patient [and caregiver]: Think about when those low or "OFF" periods have occurred over the past week. Do you usually have more problems doing things or being with people than compared to the rest of the day when you feel your medications working? Are there some things you usually do during a good period that you have trouble with or stop doing during a low period?* 0: Normal: No fluctuations or No impact by fluctuations on performance of activities or social interactions. 1: Slight: Fluctuations impact on a few activities, but during OFF, the patient usually performs all activities and participates in all social interactions that typically occur during the ON state. 2: Mild: Fluctuations impact many activities, but during OFF, the patient still usually performs all activities and participates in all social interactions that typically occur during the ON state. 3: Moderate: Fluctuations impact on the performance of activities during OFF to the point that the patient usually does not perform some activities or participate in some social interactions that are performed during ON periods. 4: Severe: Fluctuations impact on function to the point that, during OFF, the patient usually does not perform most activities or participate in most social interactions that are performed during ON periods.	☐
4.5 COMPLEXITY OF MOTOR FLUCTUATIONS Instructions to examiner: Determine the usual predictability of OFF function whether due to dose, time of day, food intake or other factors. Use the information provided by the patients and caregiver and supplement with your own observations. You will ask if the patient can count on them always coming at a special time, mostly coming at a special time (in which case you will probe further to separate slight from mild), only sometimes coming at a special time or are they totally unpredictable? Narrowing down the percentage will allow you to find the correct answer. *Instructions to patient [and caregiver]: For some patients, the low or "OFF" periods happen at certain times during day or when they do activities like eating or exercising. Over the past week, do you usually know when your low periods will occur? In other words, do your low periods always come at a certain time? Do they mostly come at a certain time? Do they only sometimes come at a certain time? Are your low periods totally unpredictable?"* 0: Normal: No motor fluctuations. 1: Slight: OFF times are predictable all or almost all of the time (> 75%). 2: Mild: OFF times are predictable most of the time (51-75%). 3: Moderate: OFF times are predictable some of the time (26-50%). 4: Severe: OFF episodes are rarely predictable. (≤ 25%).	☐

C. "OFF" DYSTONIA

4.6 PAINFUL OFF-STATE DYSTONIA

Instructions to examiner: For patients who have motor fluctuations, determine what proportion of the OFF episodes usually includes painful dystonia? You have already determined the number of hours of "OFF" time (4.3). Of these hours, determine how many are associated with dystonia and calculate the percentage. If there is no OFF time, mark 0.

Instructions to patient [and caregiver]: In one of the questions I asked earlier, you said you generally have ___ hours of low or "OFF" time when your Parkinson's disease is under poor control. During these low or "OFF" periods, do you usually have painful cramps or spasms? Out of the total ____ hrs of this low time, if you add up all the time in a day when these painful cramps come, how many hours would this make?

0: Normal: No dystonia OR NO OFF TIME.

1: Slight: < 25% of time in OFF state.

2: Mild: 26-50% of time in OFF state.

3: Moderate: 51-75% of time in OFF state.

4: Severe: > 75% of time in OFF state.

1. Total Hours Off:	_____
2. Total Off Hours w/Dystonia:	_____
3. % Off Dystonia = ((2/1)*100):	_____

Summary statement to patient: READ TO PATIENT

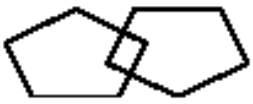
This completes my rating of your Parkinson's disease. I know the questions and tasks have taken several minutes, but I wanted to be complete and cover all possibilities. In doing so, I may have asked about problems you do not even have, and I may have mentioned problems that you may never develop at all. Not all patients develop all these problems, but because they can occur, it is important to ask all the questions to every patient. Thank you for your time and attention in completing this scale with me.

APPENDIX 12: MINI MENTAL STATE EXAMINATION

Mini-Mental State Examination (MMSE)

Patient's Name: _____ Date: _____

Instructions: Score one point for each correct response within each question or activity.

Maximum Score	Patient's Score	Questions
5		"What is the year? Season? Date? Day? Month?"
5		"Where are we now? State? County? Town/city? Hospital? Floor?"
3		The examiner names three unrelated objects clearly and slowly, then the instructor asks the patient to name all three of them. The patient's response is used for scoring. The examiner repeats them until patient learns all of them, if possible.
5		"I would like you to count backward from 100 by sevens." (93, 86, 79, 72, 65, ...) Alternative: "Spell WORLD backwards." (D-L-R-O-W)
3		"Earlier I told you the names of three things. Can you tell me what those were?"
2		Show the patient two simple objects, such as a wristwatch and a pencil, and ask the patient to name them.
1		"Repeat the phrase: 'No ifs, ands, or buts.'"
3		"Take the paper in your right hand, fold it in half, and put it on the floor." (The examiner gives the patient a piece of blank paper.)
1		"Please read this and do what it says." (Written instruction is "Close your eyes.")
1		"Make up and write a sentence about anything." (This sentence must contain a noun and a verb.)
1		"Please copy this picture." (The examiner gives the patient a blank piece of paper and asks him/her to draw the symbol below. All 10 angles must be present and two must intersect.) 
30		TOTAL

APPENDIX 13: TRAIL MAKING A & B

Trail Making Test (TMT) Parts A & B

Instructions:

Both parts of the Trail Making Test consist of 25 circles distributed over a sheet of paper. In Part A, the circles are numbered 1 – 25, and the patient should draw lines to connect the numbers in ascending order. In Part B, the circles include both numbers (1 – 13) and letters (A – L); as in Part A, the patient draws lines to connect the circles in an ascending pattern, but with the added task of alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc.). The patient should be instructed to connect the circles as quickly as possible, without lifting the pen or pencil from the paper. Time the patient as he or she connects the "trail." If the patient makes an error, point it out immediately and allow the patient to correct it. Errors affect the patient's score only in that the correction of errors is included in the completion time for the task. It is unnecessary to continue the test if the patient has not completed both parts after five minutes have elapsed.

Step 1:	Give the patient a copy of the Trail Making Test Part A worksheet and a pen or pencil.
Step 2:	Demonstrate the test to the patient using the sample sheet (Trail Making Part A – *SAMPLE*).
Step 3:	Time the patient as he or she follows the "trail" made by the numbers on the test.
Step 4:	Record the time.
Step 5:	Repeat the procedure for Trail Making Test Part B.

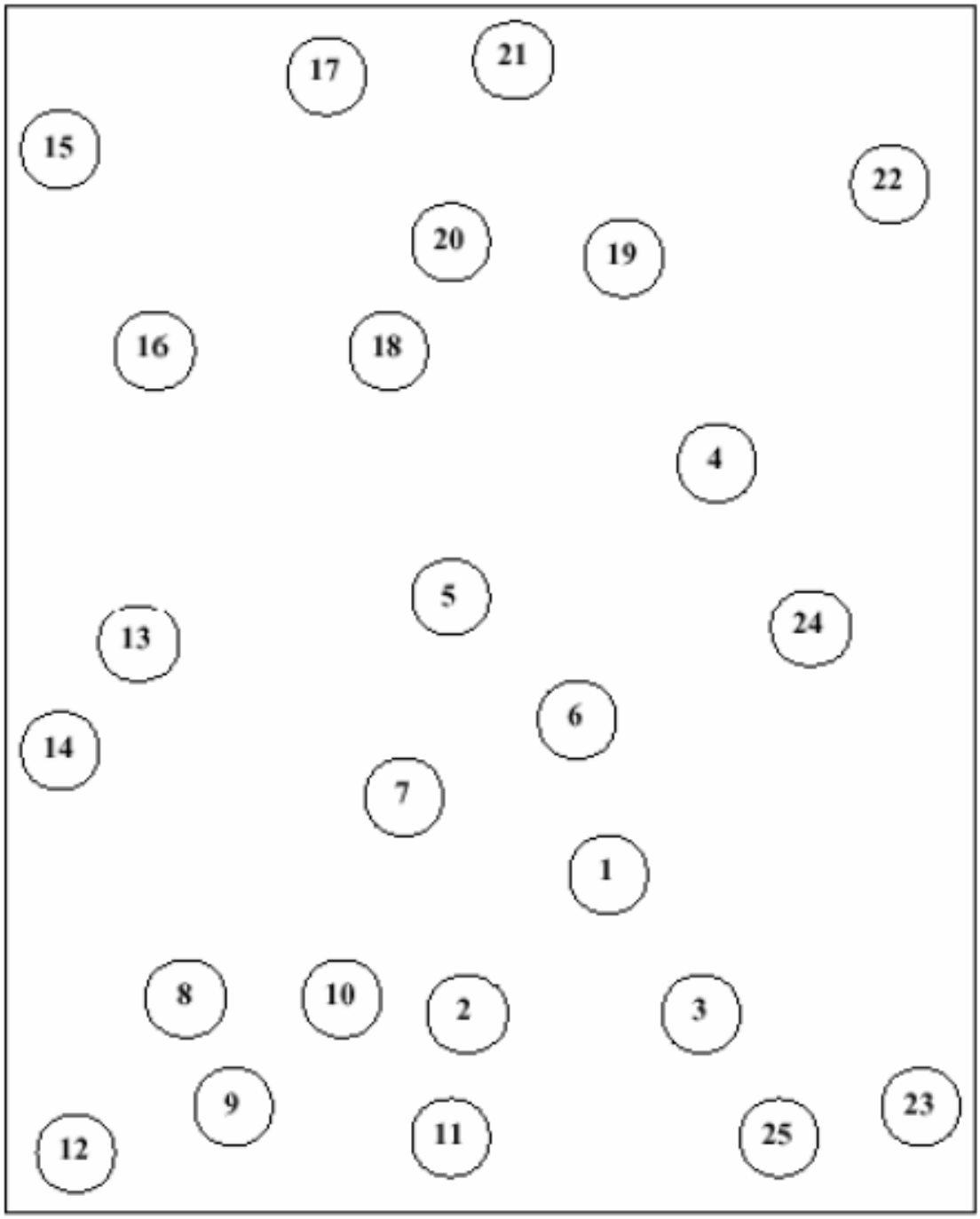
Scoring:

Results for both TMT A and B are reported as the number of seconds required to complete the task; therefore, higher scores reveal greater impairment.

	Average	Deficient	Rule of Thumb
Trail A	29 seconds	> 78 seconds	Most in 90 seconds
Trail B	75 seconds	> 273 seconds	Most in 3 minutes

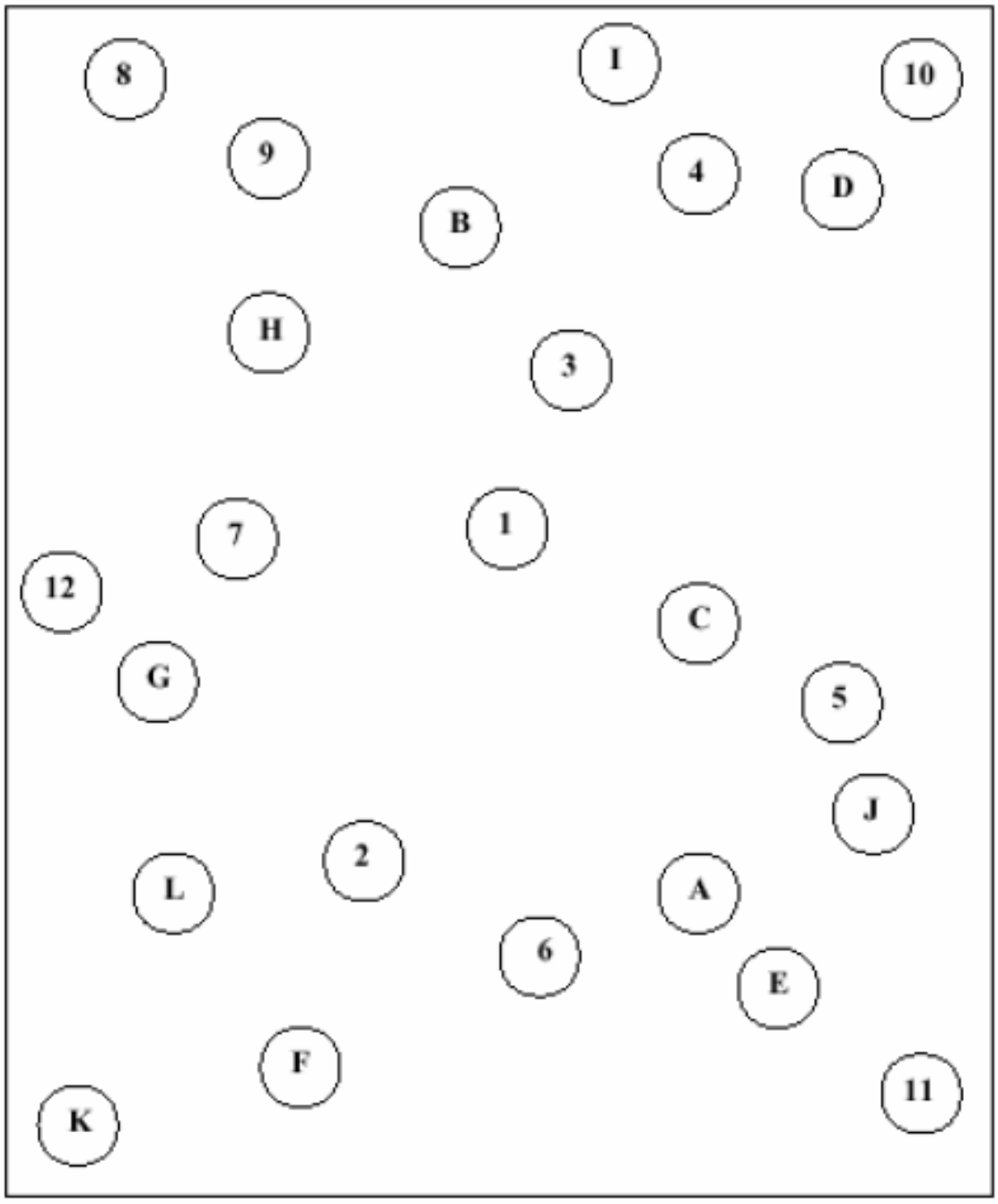
Trail Making Test Part A

Patient's Name: _____ Date: _____



Trail Making Test Part B

Patient's Name: _____ Date: _____



APPENDIX 14: UM-PDHQ

The University of Miami Parkinson's disease Hallucinations Questionnaire (UM-PDHQ)

Patient identifier: DATE:

	Question	A:Features/Comments	B:Score (circle appropriate)
Severity of hallucinations	1. Do you experience hallucinations? (Have you noticed anything unusual about your vision? Have you had any unusual visual experiences? Or ever see/hear/feel/smell/taste things that are not really there or that other people do not see?)	Type: (mark appropriate) 1. Visual 2. Auditory 3. Somatic/Cutaneous 4. Gustatory 5. Olfactory (assess each separately)	0. No hallucinations (skip to Annex) 1. One type only 2. Combination C: Not within the past month, but it has happened in the past
	2. How often do you experience hallucinations?		0 = Only a few times 1 = Occasionally (less than once a week, but continuously) 2 = Often (about once per week) 3 = Frequently (several times per week but < than once per day) 4 = Very frequently (≥once per day)
	3. On average, how long do the experiences last?		0 = Short Duration (< 1sec) 1 = Medium Duration (< 10secs) 2 = Prolonged Duration (> 10secs)
	4. Do you think what you are seeing/experiencing is real?		0 = Not real 1 = Sometimes real 2 = Always real
	5. How many types of images/sensations do you experience?		1 = One 2 = Few (2 or 3) 3 = Several (more than 3)
	6. How severe/emotionally distressing do you find these images/sensations or visions?		0 = No effect/Friendly 1 = Mildly – produce little distress 2 = Moderately – produce distress and are disturbing and disruptive 3 = Severely – very disturbing (medications may be required)
	Total Score (min = 0; max = 14)		

Comments:

	Please circle the appropriate answer and provide information	
Quality of hallucinations	7. Have you been diagnosed with any eye disease? (i.e. near or far sight problems, double vision, cataract, glaucoma, retinitis, retinal detachment, diabetic or hypertensive eye disease)	Yes (please describe) No
	8. What are your current medications?	*Complete medication data on page 4.*
	9. Was there a recent change in your treatment? Please describe.	Yes (please describe) No
	10. Was this change related to the appearance or change in the characteristics of hallucinations?	Yes No I cannot tell N/A
	11. Do you experience hallucinations while "on" or "off"?	On Off Anytime-not related to on-offs
	12. What do you normally see/feel/hear/smell/taste? If not visual describe here: Voices, Music, tastes, smells, skin related:	Not formed/cannot describe Whole Faces Fragmented faces ☐ Familiar Whole people ☐ Unfamiliar Animals Insects/reptiles Objects
	13. Is there anything you can do to make the images/sensations disappear?	Yes No
	14. At what time of the day or under which lighting conditions do you experience hallucinations	A. Specific time During the day/Bright During the night/Dark Dim B. Anytime
	15. Do the images ever make any sound or noise (for visual hallucinations)?	Yes No N/A (for non-visual hallucinations)
	16. Do images move (for visual hallucinations)?	Yes No N/A (for non-visual hallucinations)
	17. Are the images normal size?	Yes No, smaller than normal No, larger than normal N/A (for non-visual hallucinations)
	18. Are the images transparent or solid?	Transparent Solid N/A (for non-visual hallucinations)
	19. Are the images colored?	Yes No, (black and white) N/A (for non-visual hallucinations)
	20. Is the onset of hallucinations gradual or sudden?	Gradual (appear-disappear slowly) Sudden (appear-disappear suddenly) I cannot tell

APPENDIX 15: PARKINSON'S DISEASE FATIGUE SCALE (PFS-16)

Parkinson's Disease Fatigue Scale (PFS-16)

Printed below are a series of statements about fatigue and the impact that it can have.

How well do the statements describe your own feelings and experiences over the past two weeks?

Read each item and decide how much you agree or disagree with it. Then tick the appropriate box.

Tick only one box for each item and try not to miss any out.

		Strongly disagree	*Disagree*	*Do not agree or disagree*	*Agree*	*Strongly agree*
1	I have to rest during the day	☐	☐	☐	☐	☐
2	My life is restricted by fatigue	☐	☐	☐	☐	☐
3	I get tired more quickly than other people I know	☐	☐	☐	☐	☐
4	Fatigue is one of my three worst symptoms	☐	☐	☐	☐	☐
5	I feel completely exhausted	☐	☐	☐	☐	☐
6	Fatigue makes me reluctant to socialise	☐	☐	☐	☐	☐
7	It takes me longer to get things done because of fatigue	☐	☐	☐	☐	☐
8	I have a feeling of heaviness	☐	☐	☐	☐	☐
9	If I wasn't so tired I could do more things	☐	☐	☐	☐	☐
10	Everything I do is an effort	☐	☐	☐	☐	☐
11	I feel tired for much of the time	☐	☐	☐	☐	☐
12	I feel totally drained	☐	☐	☐	☐	☐
13	Fatigue makes it difficult for me to cope with everyday activities	☐	☐	☐	☐	☐
14	I feel tired even when I haven't done anything	☐	☐	☐	☐	☐
15	Because of fatigue I do less in my day than I would like	☐	☐	☐	☐	☐
16	I get so tired I want to lie down wherever I am	☐	☐	☐	☐	☐

Scoring method 1

Strong Disagree	1
Disagree	2
Neither agree nor disagree	3
Agree	4
Strongly agree	5

Scoring method 2 *

Strong Disagree	0
Disagree	0
Neither agree nor disagree	0
Agree	1
Strongly agree	1

Score of ≥8 indicates the presence of significant fatigue

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* Note: Although Scoring method 2 is easier to compute, a study of the metric properties of the PFS-16 did not recommend its use.

APPENDIX 16: PATIENT ASSESSMENT OF CONSTIPATION SYMPTOMS

PAC-SYM

This questionnaire asks you about your constipation in the **past 2 weeks.** Answer each question according to your symptoms, as accurately as possible. There are no right or wrong answers.

For each symptom below, please indicate **how severe** your symptoms have been during the **past 2 weeks.** If you have not had the symptom during the past 2 weeks, check 0. If the symptom seemed mild, check 1. If the symptom seemed moderate, check 2. If the symptom seemed severe, check 3. If the symptom seemed very severe, check 4. Please be sure to answer every question.

How severe have each of these symptoms been in the last 2 weeks	Absent	Mild	Moderate	Severe	Very Severe
	0	1	2	3	4
Discomfort in your abdomen					
Pain in your abdomen					
Bloating in your abdomen					
Stomach cramps					
Painful bowel movements					
Rectal burning during or after a bowel movement					
Incomplete bowel movement, like you didn't "finish"					
Bowel movements that were too hard					
Bowel movements that were too small					
Straining or squeezing to try to pass bowel movements					
Feeling like you have to pass a bowel movement but you couldn't (false alarm)					

APPENDIX 17: PATIENT ASSESSMENT OF CONSTIPATION QUALITY OF LIFE

PAC-QOL©

PATIENT ASSESSMENT OF CONSTIPATION

The following questions are designed to measure the impact constipation has had on your daily life over the past 2 weeks. For each question, please check one box.

The following questions ask about your symptoms related to constipation. During the past 2 weeks, to what extent or intensity have you...	Not at all 1	A little bit 2	Moderately 3	Quite a bit 4	Extremely 5
1. felt bloated to the point of bursting?	☐	☐	☐	☐	☐
2. felt heavy because of your constipation?	☐	☐	☐	☐	☐
The next few questions ask about how constipation affects your daily life. During the past 2 weeks, how much of the time have you...	None of the time 1	A little of the time 2	Some of the time 3	Most of the time 4	All of the time 5
3. felt any physical discomfort?	☐	☐	☐	☐	☐
4. felt the need to have a bowl movement but not been able to?	☐	☐	☐	☐	☐
5. been embarrassed to be with other people?	☐	☐	☐	☐	☐
6. been eating less and less because of not being able to have bowel movements?	☐	☐	☐	☐	☐

The next few questions ask about how constipation affects your daily life. During the past 2 weeks, to what extent or intensity have you...	Not at all 0	A little bit 1	Moderately 2	Quite a bit 3	Extremely 4
7. had to be careful about what you eat?	☐	☐	☐	☐	☐
8. had a decreased appetite?	☐	☐	☐	☐	☐
9. been worried about not being able to choose what you eat (for example, at a friend's house)?	☐	☐	☐	☐	☐
10. been embarrassed about staying in the bathroom for so long when you were away from home?	☐	☐	☐	☐	☐
11. been embarrassed about having to go to the bathroom so often when you were away from home?	☐	☐	☐	☐	☐
12. been worried about having to change your daily routine (for example, traveling, being away from home)?	☐	☐	☐	☐	☐
The next few questions ask about your feelings related to constipation. During the past 2 weeks, how much of the time have you...	None of the time 1	A little of the time 2	Some of the time 3	Most of the time 4	All of the time 5
13. felt irritable because of your condition?	☐	☐	☐	☐	☐
14. been upset by your condition?	☐	☐	☐	☐	☐
15. felt obsessed by your condition?	☐	☐	☐	☐	☐
16. felt stressed by your condition?	☐	☐	☐	☐	☐
17. felt less self-confident because of your condition?	☐	☐	☐	☐	☐
18. felt in control of your situation?	☐	☐	☐	☐	☐

The next questions ask about your feelings related to constipation. During the past 2 weeks, to what extent or intensity have you...	Not at all 0	A little bit 1	Moderately 2	Quite a bit 3	Extremely 4
19. been worried about not knowing when you are going to be able to have a bowel movement?	☐	☐	☐	☐	☐
20. been worried about not being able to have a bowel movement?	☐	☐	☐	☐	☐
21. been more and more bothered by not being able to have a bowel movement?	☐	☐	☐	☐	☐

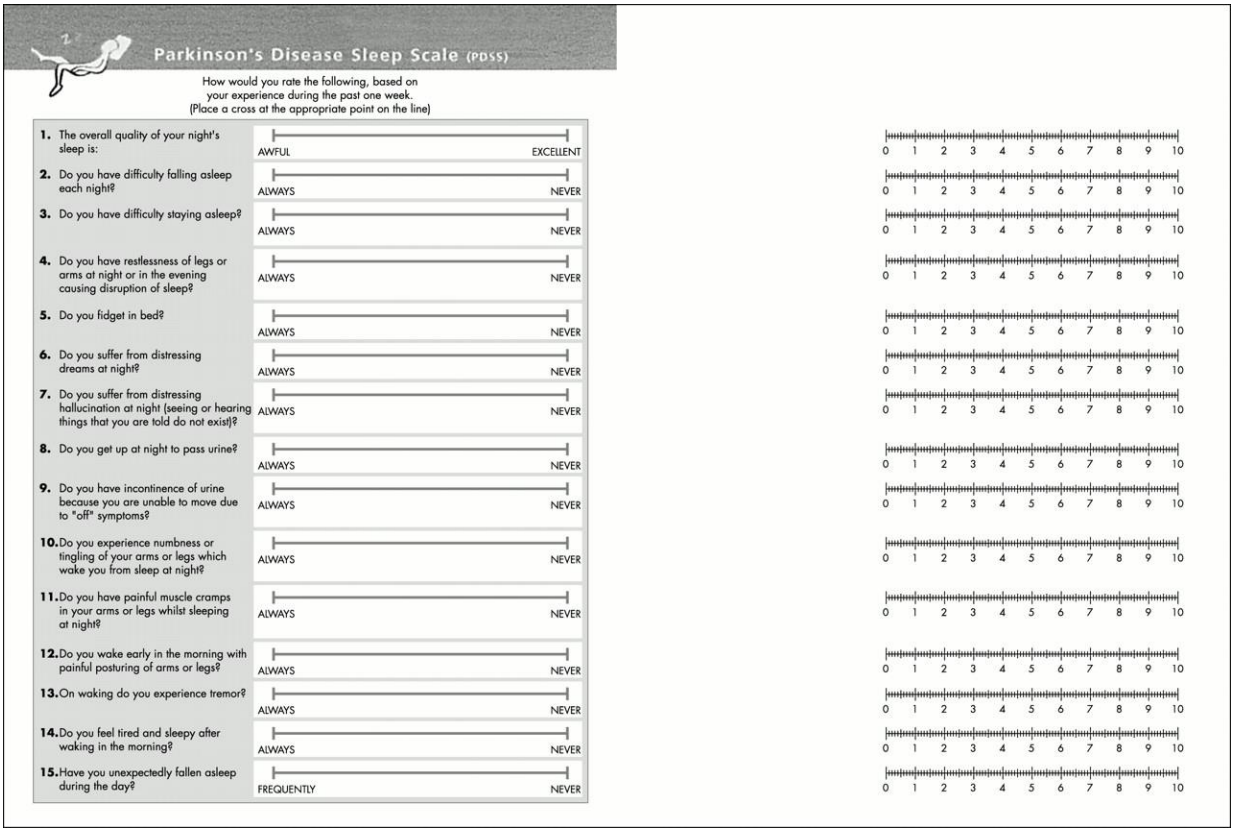
The next questions ask about your life with constipation. During the past 2 weeks, how much of the time have you...	None of the time 1	A little of the time 2	Some of the time 3	Most of the time 4	All of the time 5
22. been worried that your condition will get worse?	☐	☐	☐	☐	☐
23. felt that your body was not working properly?	☐	☐	☐	☐	☐
24. had fewer bowel movements than you would like?	☐	☐	☐	☐	☐

The next questions ask about your degree of satisfaction related to constipation. During the past 2 weeks, to what extent or intensity have you been...	Not at all 0	A little bit 1	Moderately 2	Quite a bit 3	Extremely 4
25. satisfied with how often you have a bowel movement?	☐	☐	☐	☐	☐
26. satisfied with the regularity of your bowel movements?	☐	☐	☐	☐	☐
27. satisfied with the time it takes for food to pass through the intestines?	☐	☐	☐	☐	☐
28. satisfied with your treatment?	☐	☐	☐	☐	☐

APPENDIX 18: REM SLEEP BEHAVIOR DISORDER SCREENING QUESTIONNAIRE

1. I sometimes have very vivid dreams. yes/no
2. My dreams frequently have an aggressive or action-packed content. yes/no
3. The dream contents mostly match my nocturnal behavior. yes/no
4. I know that my arms or legs move when I sleep. yes/no
5. It thereby happened that I (almost) hurt my bed partner or myself. yes/no
6. I have or had the following phenomena during my dreams:
    - 6.1. Speaking, shouting, swearing, laughing loudly yes/no
    - 6.2. Sudden limb movements, “fights” yes/no
    - 6.3. Gestures, complex movements, that are useless during sleep, e.g., to wave, to salute, to frighten mosquitoes, fall off the bed yes/no
    - 6.4. Things that fell down around the bed, e.g., bedside lamp, book, glasses yes/no
7. It happens that my movements awake me. Yes/no
8. After awakening I mostly remember the content of my dreams well. Yes/no
9. My sleep is frequently disturbed. Yes/no
10. I have/had a disease of the nervous system (e.g., stroke, head trauma, Parkinsonism, RLS, narcolepsy, depression, epilepsy, inflammatory disease of the brain), which? yes/no

APPENDIX 19: PARKINSON'S DISEASE SLEEP SCALE



Parkinson's Disease Sleep Scale (PDSS)

How would you rate the following, based on your experience during the past one week. (Place a cross at the appropriate point on the line)

1.	The overall quality of your night's sleep is:	AWFUL	EXCELLENT
2.	Do you have difficulty falling asleep each night?	ALWAYS	NEVER
3.	Do you have difficulty staying asleep?	ALWAYS	NEVER
4.	Do you have restlessness of legs or arms at night or in the evening causing disruption of sleep?	ALWAYS	NEVER
5.	Do you fidget in bed?	ALWAYS	NEVER
6.	Do you suffer from distressing dreams at night?	ALWAYS	NEVER
7.	Do you suffer from distressing hallucination at night (seeing or hearing things that you are told do not exist)?	ALWAYS	NEVER
8.	Do you get up at night to pass urine?	ALWAYS	NEVER
9.	Do you have incontinence of urine because you are unable to move due to "off" symptoms?	ALWAYS	NEVER
10.	Do you experience numbness or tingling of your arms or legs which wake you from sleep at night?	ALWAYS	NEVER
11.	Do you have painful muscle cramps in your arms or legs whilst sleeping at night?	ALWAYS	NEVER
12.	Do you wake early in the morning with painful posturing of arms or legs?	ALWAYS	NEVER
13.	On waking do you experience tremor?	ALWAYS	NEVER
14.	Do you feel tired and sleepy after waking in the morning?	ALWAYS	NEVER
15.	Have you unexpectedly fallen asleep during the day?	FREQUENTLY	NEVER

0 1 2 3 4 5 6 7 8 9 10