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Annals of Internal Medicine ORIGINAL RESEARCH

Oral ENT-01 Targets Enteric Neurons to Treat Constipation in Parkinson Disease

A Randomized Controlled Trial

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Background: Parkinson disease (PD) is associated with α-synuclein (αS) aggregation within enteric neurons. ENT-01 inhibits the formation of αS aggregates and improved constipation in an open-label study in patients with PD.

Objective: To evaluate the safety and efficacy of oral ENT-01 for constipation and neurologic symptoms in patients with PD and constipation.

Design: Randomized, placebo-controlled phase 2b study. (ClinicalTrials.gov: NCT03781791)

Setting: Outpatient.

Patients: 150 patients with PD and constipation.

Intervention: ENT-01 or placebo daily for up to 25 days. After baseline assessment of constipation severity, daily dosing was escalated to the prokinetic dose, the maximum dose (250 mg), or the tolerability limit, followed by a washout period.

Measurements: The primary efficacy end point was the number of complete spontaneous bowel movements (CSBMs) per week. Neurologic end points included dementia (assessed using the Mini-Mental State Examination [MMSE]) and psychosis (assessed using the Scale for the Assessment of Positive Symptoms adapted for PD [SAPS-PD]).

Results: The weekly CSBM rate increased from 0.7 to 3.2 in the ENT-01 group versus 0.7 to 1.2 in the placebo group ($P < 0.001$). Improvement in secondary end points included SBMs ($P = 0.002$), stool consistency ($P < 0.001$), ease of passage ($P = 0.006$), and laxative use ($P = 0.041$). In patients with dementia, MMSE scores improved by 3.4 points 6 weeks after treatment in the ENT-01 group ($n = 14$) versus 2.0 points in the placebo group ($n = 14$). Among patients with psychosis, SAPS-PD scores improved from 6.5 to 1.7 six weeks after treatment in the ENT-01 group ($n = 5$) and from 6.3 to 4.4 in the placebo group ($n = 6$). ENT-01 was well tolerated, with no deaths or drug-related serious adverse events. Adverse events were predominantly gastrointestinal, including nausea (34.4% [ENT-01] vs. 5.3% [placebo]; $P < 0.001$) and diarrhea (19.4% [ENT-01] vs. 5.3% [placebo]; $P = 0.016$).

Limitation: Longer treatment periods need to be investigated in future studies.

Conclusion: ENT-01 was safe and significantly improved constipation.

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For author, article, and disclosure information, see end of text.
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Parkinson disease (PD) is a progressive neurodegenerative disorder caused by accumulation of α-synuclein (αS) in the enteric nervous system (ENS) (1, 2) and the central nervous system (CNS) (3). Although motor symptoms are required for a diagnosis of PD (4), nonmotor symptoms cause significant symptom burden and therapeutic challenges (5). These symptoms include constipation (6, 7), disturbances in sleep architecture (8, 9), cognitive dysfunction (10), psychosis (11, 12), and depression (13), all of which result from impaired function of neural pathways not restored by replacement of dopamine. Epidemiologic and preclinical studies suggest that aggregates of αS formed within the ENS might cause gastrointestinal (GI) dysmotility (14) and underlie the cause of the CNS component of PD (2).

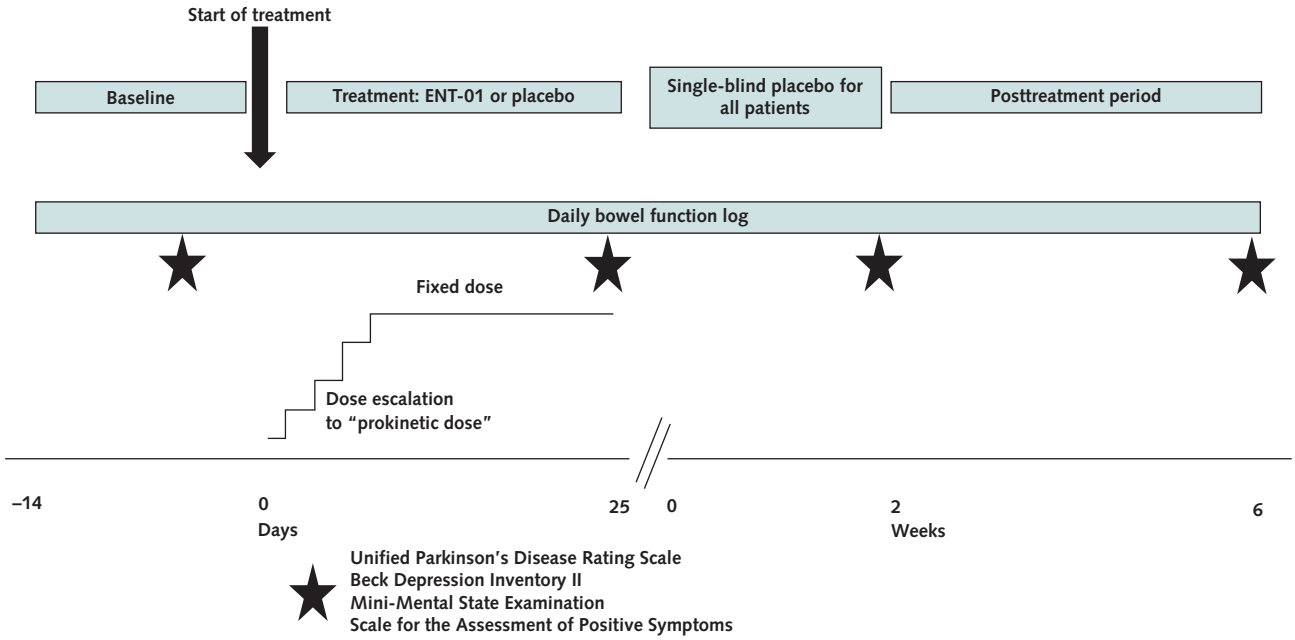
Constipation affects more than 60% of persons with PD, and in most, the condition is chronic, severe, and unresponsive to standard therapy (6, 15), in large part due to the underlying functional disruption of the ENS. In addition to causing a major economic burden, constipation significantly affects the quality of life of persons with PD, contributing to social isolation and depression. Furthermore, the severity of the symptoms correlates negatively with patient-reported quality of life.

A strategy that targets neurotoxic aggregates of αS in the GI tract represents a novel approach to treatment of PD. Such an approach may restore the function of

See also:

Web-Only
Supplement

Figure 1. Experimental design.



enteric neurons, improve neural signaling between the gut and the brain, and prevent the production and accumulation of αS in the ENS and CNS. Such actions may slow progression of neurologic symptoms in addition to restoring GI function.

ENT-01 (squalamine phosphate) displaces αS that is bound electrostatically to nerve cell membranes and has been shown to prevent aggregation of αS monomers both in vitro and in a *Caenorhabditis elegans* model of PD in vivo (16). In mouse models of PD, ENT-01 stimulates enteric neurons and reverses GI dysmotility (17).

An open-label study (RASMET) involving 50 patients with PD and constipation showed that ENT-01 rapidly and safely improves bowel function. Neuropsychiatric symptoms also improved, suggesting that directly targeting enteric αS might be beneficial in PD. Systemic absorption of the compound was negligible, suggesting that ENT-01 improved bowel function by acting locally in the GI tract (18).

Given these findings, we conducted a randomized, placebo-controlled phase 2b study (KARMET) involving 150 patients with PD to determine the safety and efficacy of up to 25 days of ENT-01 treatment for constipation and neurologic symptoms.

METHODS

Study Design

We conducted a randomized, double-blind, placebo-controlled, escalating-dose study to evaluate the safety and efficacy of orally administered ENT-01 for constipation and neurologic symptoms in patients with PD and constipation. The protocol was approved by the institutional review board at each participating center, and patients provided written informed consent. The original and final study protocols and the statistical analysis plan are available at Annals.org.

Inclusion and Exclusion Criteria

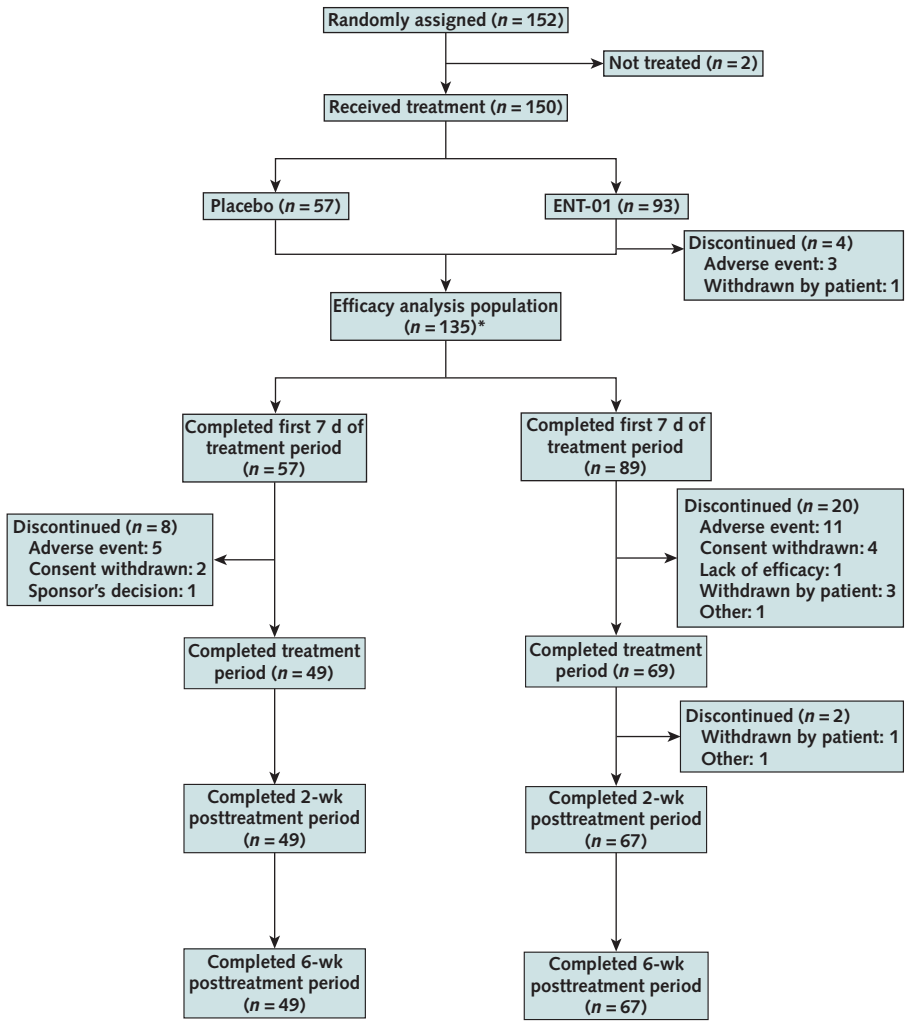
Adults aged 18 to 90 years were eligible to participate if they had a diagnosis of PD and constipation that was not attributable to another cause. The disease was diagnosed by a movement disorder specialist according to the UK Parkinson's Disease Society Brain Bank criteria (19). Patients were deemed to have constipation if they had fewer than 3 complete spontaneous bowel movements (CSBMs) per week and satisfied the Rome IV criteria for functional constipation (20). The criteria require 2 or more of the following in at least 25% of defecations: straining, lumpy or hard stools, a sensation of incomplete evacuation, a sensation of anorectal obstruction or blockage, or manual maneuvers to facilitate defecation.

The key exclusion criteria were the presence of a potential safety risk for a prokinetic agent, such as underlying structural bowel disease; concomitant medications or conditions that may have confounded end point measures; risk for nonadherence; and participation in other trials (see the final study protocol).

Trial Procedures

Patients underwent a 14-day run-in to assess constipation severity using a validated daily bowel function log (21) to establish the baseline number of CSBMs per week. Laxatives were withdrawn at the beginning of the baseline period and replaced with "rescue medication" (bisacodyl tablets, suppositories, and saline [Fleet] enemas) to be taken every 3 days if there were no bowel movements. Any bowel movement within 24 hours of rescue medication use was not counted. Rescue medication use

Figure 2. Patient disposition.



* Excludes 11 patients (6 in the ENT-01 group and 5 in the placebo group) from 2 sites, based on an audit of the sites.

continued until standard laxatives were reinstated at the end of the washout period.

Information about occurrence, timing, consistency, ease of passage, and completeness of bowel movements was recorded daily using an Apple iPad. If information was not entered by 7:00 p.m., the patient received 3 notifications between 7:00 p.m. and 11:59 p.m. The log for any given day was "locked" at midnight.

Patients with an average of fewer than 3 CSBMs per week were randomly assigned via a computerized interactive response technology to ENT-01 or placebo in a 3:1 ratio and stratified to 1 of 2 starting doses according to constipation severity. Patients with 0 to 0.9 CSBM per week received 150 mg of ENT-01 daily (or 6 placebo pills), and those with 1.0 to 2.9 CSBMs per week received 75 mg of ENT-01 daily (or 3 placebo pills). Dosing was escalated in both groups by 25 mg (or 1 placebo pill) every 3 days to a dose that produced a CSBM within 24 hours of administration on at least 2 of 3 days (the "prokinetic" dose), to the maximum dose of 250 mg (or 10 placebo pills), or to the tolerability limit. Dosing was fixed for the remainder of the 25 days. After the "fixed-dose period," all patients were switched to placebo (with participants blinded) for 2 weeks, followed by a 4-week washout (**Figure 1**). A computer generated the randomization, and allocation was concealed from all patients and study staff.

Study End Points

The safety end point was assessed via adverse events, including vomiting, diarrhea, abdominal pain, and dizziness. The primary efficacy end point was the number of CSBMs during the fixed-dose period. Two definitions of CSBM were used during the study to enhance the ability of patients to understand the concept (**Supplement Table** 1, available at Annals.org). With the exception of the sensitivity analyses presented in **Supplement Table** 1, all CSBM analyses were based on definition 2. Secondary efficacy end points included the proportion of patients with an increase of 1 or more CSBMs per week or an absolute

number of 3 or more CSBMs per week, the change from baseline in the number of SBMs per week, stool consistency (Bristol Stool Form Scale) (22, 23), ease of passage (Ease of Evacuation Scale) (24), rescue medication use, symptoms (Patient Assessment of Constipation Symptoms [PAC-SYM]), and quality of life (Patient Assessment of Constipation Quality of Life [PAC-QOL]) related to bowel function (25, 26).

Exploratory Neurologic End Points

Symptoms of PD were assessed using the Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) (19), depression was assessed using the Beck Depression Inventory II (27), cognition was assessed using the Mini-Mental State Examination (MMSE) (28), and psychosis was assessed using the Scale for the Assessment of Positive Symptoms adapted for PD (SAPS-PD) (29). Assessments were done at baseline, the end of the treatment period, and 2 and 6 weeks after treatment discontinuation. Patients with dementia (baseline MMSE score ≤26) and those with psychosis (baseline SAPS-PD score ≥4) were analyzed separately.

Statistical Analysis

The study had 90% power to detect a difference in CSBM rates of 1.9 per week, based on a 2-sided *t* test at a significance level of 0.05. On the basis of the RASMET study, we assumed 64 (89%) of 72 patients would be evaluable, an increase in the mean number of CSBMs per week of 2.4 in the ENT-01 group and 0.5 in the placebo group, and an SD of 2.0.

Based on the prespecified interim analysis of the initial cohort to appraise study power, the mean changes in the number of CSBMs were 3.6 (SD, 4.11) and 1.3 (SD, 2.38) per week with ENT-01 and placebo, respectively. Therefore, based on a 2-sided *t* test, the study required 80 additional patients randomly assigned in a 1:1 ratio for a conditional power of 90%.

For the primary outcome (CSBM rate), we analyzed the difference between the groups by using 2 approaches. First, a negative binomial mixed model was used to control for patients' repeated measures across 3 periods (the end of treatment, 2 weeks after treatment, and 6 weeks after treatment). Second, as described in the protocol, we analyzed the change from baseline in the CSBM rate using analysis of covariance (ANCOVA), with treatment as a factor and the baseline CSBM rate as a covariate.

The continuous secondary and exploratory efficacy end points were analyzed by an ANCOVA model, with treatment and baseline CSBM strata as factors and the corresponding baseline values as covariates. The Fisher exact test was used to compare adverse event rates.

The primary efficacy analyses included patients who completed at least 7 days of treatment ($n = 135$). Data from 2 sites ($n = 11$) were excluded on the basis of an independent review and a quality audit of stool diary data (Figure 2). Results of a sensitivity analysis that included these patients are shown in Supplement Table 2 (available at Annals.org).

We assumed bowel movement and laxative use values of 0 if the corresponding daily question was unanswered; missing values were not imputed for other assessments. There were no adjustments for multiple comparisons. Post hoc analyses were done on the subset of patients with neuropsychiatric symptoms at baseline.

Statistical analyses were performed using SAS, version 9.4 (SAS Institute).

Table 1. Baseline Characteristics of Patients

Characteristic	ENT-01 (*n* = 93)	Placebo (*n* = 57)	Total (*n* = 150)
Sex, *n* (%)			
Male	55 (59.1)	33 (57.9)	88 (58.7)
Female	38 (40.9)	24 (42.1)	62 (41.3)
White race, *n* (%)	87 (93.5)	52 (91.2)	139 (92.7)
Age, *y*			
Mean (SD)	67.5 (8.75)	70.0 (6.66)	68.5 (8.09)
Range	44–86	49–79	44–86
Age at PD diagnosis, *y*			
Mean (SD)	60.8 (9.29)	62.2 (8.22)	61.3 (8.90)
Range	37.9–76.9	34.4–77.9	34.4–77.9
Duration of PD, *y*			
Mean (SD)	7.4 (5.81)	8.4 (6.40)	7.8 (6.03)
Range	0.4–29.2	0.2–35.2	0.2–35.2
Duration of constipation, *y*			
Mean (SD)	10.2 (10.94)	16.6 (19.86)	12.7 (15.25)
Range	0.5–60.2	0.2–77.7	0.2–77.7
MDS-UPDRS score			
Mean (SD)	53.5 (25.09)	58.5 (25.85)	55.4 (25.41)
Range	13.0–125.0	15–127	13.0–127.0
Hoehn and Yahr stage			
Mean (SD)	1.9 (0.71)	2.3 (0.64)	2.1 (0.70)
Range	0–4.0	1.0–5.0	0–5.0
Constipation severity*, *n* (%)			
0–0.9 CSBMs/wk	41 (44.1)	31 (53.4)	72 (47.7)
1.0–2.9 CSBMs/wk	52 (55.9)	26 (44.8)	78 (51.7)

CSBM = complete spontaneous bowel movement; MDS-UPDRS = Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale; PD = Parkinson disease.
* Baseline value is the average number of CSBMs per week calculated at the end of the 2-week run-in period.

Role of the Funding Source

Enterin, Inc., funded the conduct of this research and was responsible for the study design, data collection, and analysis, with input from investigators and authors as noted. Enterin participated in the interpretation of data, the writing of the report, and the decision to submit the manuscript for publication, along with investigators and authors as noted. An independent data safety monitoring board reviewed data quality and evidence for treatment harm.

RESULTS

Patient Disposition and Characteristics

Between January 2019 and December 2021, a total of 150 patients were dosed. Baseline characteristics were similar between groups (Table 1). Daily diary adherence

Table 2. All Adverse Events and Serious Adverse Events (n = 150)*

Event	Patients, *n* (%)		*P* Value
	ENT-01 (*n* = 93)	Placebo (*n* = 57)	
Death	0	0	1.00
Any serious adverse event	3 (3.2)	1 (1.8)	1.00
Drug-related serious adverse event	0	0	1.00
Any adverse event	61 (65.6)	27 (47.4)	0.040†
Dose-limiting toxicity	23 (28)	4 (10)	0.008†
Gastrointestinal			
Nausea	32 (34.4)	3 (5.3)	<0.001†
Diarrhea	18 (19.4)	3 (5.3)	0.016†
Vomiting	8 (8.6)	0	0.024†
Dyspepsia	1 (1.1)	0	1.00
Abdominal pain	7 (7.5)	3 (5.3)	0.74
Abdominal distension	6 (6.5)	3 (5.3)	0.72
Flatulence	5 (5.4)	1 (1.8)	0.41
Hemorrhoids	0	1 (1.8)	0.38
Rectal hemorrhage	0	1 (1.8)	0.38
Fecal incontinence	1 (1.0)	0	1.00
Rectal pain	1 (1.0)	0	1.00
Fecaloma	1 (1.0)	0	1.00
Nongastrointestinal			
Dizziness	7 (7.5)	1 (1.8)	0.156
Falls	4 (4.3)	1 (1.8)	0.65
Hip fracture	0	1 (1.8)	0.38
Knee surgery	1 (1.0)	0	1.00
Cholelithiasis/cholecystectomy	1 (1.0)	0	1.00
Infection	9 (9.7)	7 (12.3)	0.60
Urinary incontinence	2 (2.2)	2 (3.5)	0.63
Retinal vein occlusion	0	1 (1.8)	0.38
Hypokalemia	1 (1.0)	0	1.00

* Patients who ever had an event are included rather than the number of events, regardless of whether the event was related to treatment.
† Statistically significant.

was more than 90% during the treatment period in both groups. The duration of constipation was slightly longer in placebo recipients, and a larger proportion had 0 to 0.9 CSBM per week at baseline; these imbalances were found not to have a significant effect on the results of the primary efficacy analysis. Therapy was discontinued before treatment completion in 24 of 93 (25.8%) patients receiving ENT-01 and 8 of 57 (14.1%) receiving placebo (**Supplement Table** 3, available at Annals.org). Discontinuations in the treatment group were mostly due to nausea or diarrhea. A majority of patients were White men. The average PD duration was 7.8 years, and the average constipation duration was 12.7 years.

Safety

There were no deaths or drug-related serious adverse events (**Table 2**). Most adverse events were confined to the GI tract. The most common treatment-emergent adverse event was nausea, which occurred in 32 of 93 (34.4%) patients in the ENT-01 group versus 3 of 57 (5.3%) in the placebo group ($P < 0.001$). Diarrhea occurred in 18 of 93 (19.4%) patients in the ENT-01 group versus 3 of 57 (5.3%) in the placebo group ($P = 0.016$). Neither nausea nor diarrhea was dose-related, but they were clustered around the starting doses of 75 and 150 mg, suggesting that they were related to treatment initiation (**Supplement Table** 4, available at Annals.org). Diarrhea responded to dose reduction; nausea tended to diminish after 2 to 3 days and improved when medication was taken with 8 oz of water and/or a light breakfast.

There were no clinically significant effects of ENT-01 on QTc or other electrocardiographic parameters.

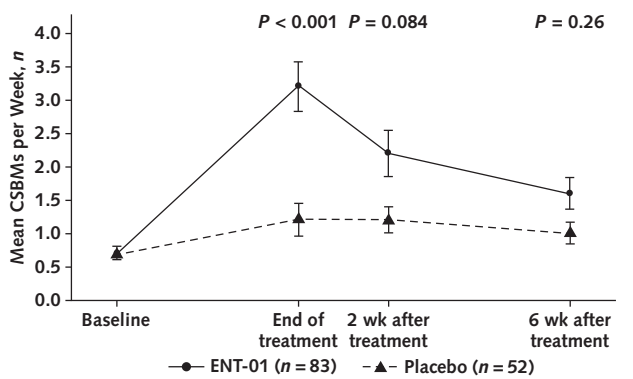
Efficacy

Effects on Bowel Function

The mean baseline CSBM rate was 0.7 per week in both groups. Mean treatment duration was 20.8 days (range, 1 to 28 days) in the ENT-01 group and 21.8 days (range, 1 to 26 days) in the placebo group. Duration of dose escalation was 12.6 days (range, 1 to 21 days), and duration of the fixed dose was 9.1 days (range, 7 to 23 days) (**Supplement Table** 5, available at Annals.org). Roughly 25% of patients reached the prokinetic dose between 75 and 125 mg, compared with 75% who required 150 to 250 mg (**Supplement Table** 6, available at Annals.org).

The CSBM rates for both groups across the 3 periods are shown in **Figure 3** and **Table 3**. The CSBM rates were 3.2 per week in the ENT-01 group and 1.2 per week in the placebo group during the fixed-dose period (rate ratio, 2.78 [95% CI, 1.61 to 4.81]; $P < 0.001$). Two weeks beyond treatment, the CSBM rates were 2.2 per week in the ENT-01 group and 1.2 per week in the placebo group (rate ratio, 1.65 [CI, 0.93 to 2.93]; $P = 0.084$). Results using ANCOVA as specified in the protocol (**Supplement Table** 7, available at Annals.org), results using 2 different definitions of CSBM (**Supplement Table** 1), and results of the sensitivity analyses (**Supplement Table** 2) were similar. The responder rate increased in a dose-dependent fashion, from 7.2% at 75 mg to 56.6% at 250 mg in the ENT-01 group and from 3.2% to 28.8% in the placebo group ($P = 0.002$) (**Supplement Figure** 1, available at Annals.org). In the ENT-01 group, the dose required for a functional bowel response was patient-specific and varied from 75 to 250 mg. The proportion of patients with 0 to 0.9 CSBM per week who were taking at least 200 mg at a fixed dose was 70.0%, compared with

Figure 3. Weekly CSBM rates at each time point.



Error bars represent SEs. All patients who completed ≥7 days of treatment were included in the analysis (n = 135). P values are based on negative binomial regression. CSBM = complete spontaneous bowel movement.

Table 3. Primary Analysis of CSBMs Across Study Periods

Time Point	CSBMs per Week (SD), *n*		Rate Ratio* (95% CI)	*P* Value	Rate Difference* (95% CI)
	ENT-01	Placebo			
End of treatment	3.2 (3.37)	1.2 (1.73)	2.78 (1.61 to 4.81)	<0.001	1.81 (0.83 to 2.80)
2 wk after treatment	2.2 (2.65)	1.2 (1.33)	1.65 (0.93 to 2.93)	0.084	0.63 (−0.07 to 1.32)
6 wk after treatment	1.6 (1.89)	1.0 (1.02)	1.36 (0.79 to 2.37)	0.26	0.30 (−0.21 to 0.81)

CSBM = complete spontaneous bowel movement.
* A negative binomial regression model was used for CSBMs across the 3 periods, with control for repeated measures. Rate ratios and rate differences were estimated from the negative binomial model. The analysis included patients who completed ≥7 days of treatment (n = 135).

49.3% in those with 1.0 to 2.9 CSBMs per week (P = 0.022). Secondary bowel end points improved significantly in the ENT-01 group, including SBMs (P = 0.002), stool consistency (P < 0.001), ease of passage (P = 0.006), and laxative use (P = 0.041) (**Table 4**; **Supplement Table** 7). PAC-SYM and PAC-QOL scores did not differ significantly between groups.

Effects on Neuropsychiatric Symptoms

Eleven patients had hallucinations at baseline (SAPS-PD score ≥4). The SAPS-PD was administered at each subsequent visit in this subgroup. In the ENT-01 group (n = 5), the mean SAPS-PD score was 6.5 (range, 5 to 11) at baseline, 1.8 (range, 0 to 4) at the end of treatment, and 1.7 (range, 0 to 4) 2 weeks after the end of treatment. This improvement was maintained 6 weeks after the end of treatment. In the placebo group (n = 6), the mean SAPS-PD score was 6.3 (range, 4 to 12) at baseline, 3.4 (range, 0 to 9) at the end of treatment, 4.0 (range, 0 to 9) 2 weeks after the end of treatment, and 4.4 (range, 0 to 10) 6 weeks after the end of treatment (**Supplement Figure** 2, available at Annals.org).

Twenty-eight patients had dementia at baseline (MMSE score ≤26). In the ENT-01 group (n = 14), mean MMSE scores were 24.1 at baseline, 26.5 at the end of treatment, 26.9 two weeks after the end of treatment, and 27.5 six weeks after the end of treatment. Mean scores in the placebo group (n = 14) were 24.8 at baseline, 25.9 at the end of treatment, 25.7 two weeks after the end of treatment, and 26.8 six weeks after the end of treatment (**Supplement Figure** 3, available at Annals.org). Differences in MMSE score between ENT-01 and placebo for the entire cohort (n = 135) were significant 6 weeks after treatment discontinuation (P = 0.03) (**Supplement Figure** 4 and **Supplement Table** 8, available at Annals.org).

There was no worsening of motor function (**Supplement Table** 8). Motor score on the MDS-UPDRS correlated with baseline constipation severity as measured by weekly CSBM rate (P = 0.001), and improvement in MDS-UPDRS score at the end of treatment was associated with improvement in CSBM rate (P = 0.001) (**Supplement Figure** 5, available at Annals.org).

DISCUSSION

In this randomized, placebo-controlled study involving 150 patients with PD and constipation, we show that orally administered ENT-01 is safe and that it rapidly normalizes bowel function in a dose-dependent fashion, with an effect that seems to persist for several weeks beyond the treatment period. This suggests that the ENS is not irreversibly damaged in PD, even though the long-term constipation might suggest otherwise.

Adverse events were largely confined to the GI tract, supporting the local action of ENT-01. Nausea and diarrhea were the most common adverse events, both of which tended to occur at treatment initiation. In future studies, starting at lower doses, escalating more slowly, and administering an antiemetic with antiserotonergic effects (such as ondansetron) at treatment initiation may reduce the frequency of both symptoms and reduce dropout.

This study replicates findings from an earlier open-label study (18), which showed that ENT-01 improves bowel function by acting locally on the GI tract, as supported by the oral bioavailability of less than 0.3%. As in the current study, the effect on bowel function persisted beyond treatment (18).

Bowel dysmotility in PD is believed to result from the functional disturbance of enteric neurons caused by the presence of intraneuronal membrane-bound aggregates of αS (1) rather than as a consequence of neuronal loss (14). ENT-01, an aminosterol of vertebrate origin, has been shown to displace toxic αS aggregates from neuronal membranes and inhibit their formation from αS monomers (16). In a PD mouse model, ENT-01 had a robust prokinetic effect that was not accompanied by an increase in stool water content, supporting a mechanism involving primary neuronal stimulation (17). The excitability of the intrinsic primary afferent neuron, a key neuron involved in gut motility, was significantly blunted in the mouse model and was restored to normal activity almost immediately by exposure to ENT-01 (17). Organized peristaltic waves resumed, and constipation was corrected (17). In addition, afferent vagal firing from the ENS directed to the brain increases, and the stimulated wave forms exhibit a serotonergic pattern (30). Using whole-brain c-Fos imaging in wild-type mice, we recently showed that 2 hours after ingestion of ENT-01, excitation was observed in the nucleus tractus solitarius (the primary sensory nucleus of the vagus) and in paraventricular thalamic nuclei (Video and **Supplement Figure** 6, available at Annals.org), regions that are heavily implicated in arousal and attention (and consequently cognition) and are reciprocally connected to the suprachiasmatic nuclei that control circadian rhythm. This may

Table 4. Effects on Bowel Function (n = 135)

Outcome	Mean Value (±SE)		Treatment Difference (95% CI)*	*P* Value
	Baseline	End of Treatment		
CSBMs, *n*†				
ENT-01	0.7 ± 0.09	3.2 ± 0.37	1.9 (0.94 to 2.93)	<0.001
Placebo	0.7 ± 0.10	1.2 ± 0.24		
SBMs, *n*†				
ENT-01	4.0 ± 0.42	6.7 ± 0.54	2.2 (0.82 to 3.63)	0.002
Placebo	4.3 ± 0.49	4.6 ± 0.54		
Consistency‡				
ENT-01	2.9 ± 0.15	4.4 ± 0.18	1.4 (0.88 to 1.89)	<0.001
Placebo	3.0 ± 0.14	3.0 ± 0.17		
Ease of passage§				
ENT-01	3.3 ± 0.07	4.0 ± 0.09	0.4 (0.11 to 0.67)	0.006
Placebo	3.5 ± 0.08	3.6 ± 0.09		
Laxative use, *n*				
ENT-01	0.7 ± 0.11	0.5 ± 0.17	−0.5 (−1.02 to −0.02)	0.041
Placebo	0.6 ± 0.18	0.9 ± 0.27		
PAC-SYM score				
ENT-01	1.4 ± 0.06	1.0 ± 0.07	−0.1 (−0.28 to 0.10)	0.35
Placebo	1.5 ± 0.09	1.1 ± 0.10		
PAC-QOL score				
ENT-01	1.3 ± 0.06	1.0 ± 0.06	0.0 (−0.19 to 0.10)	0.54
Placebo	1.4 ± 0.08	1.2 ± 0.07		

CSBM = complete spontaneous bowel movement; PAC-QOL = Patient Assessment of Constipation Quality of Life; PAC-SYM = Patient Assessment of Constipation Symptoms; SBM = spontaneous bowel movement.
* Analysis of covariance least-squares means and corresponding 95% CIs are presented for the difference in the change from baseline scores.
† Weekly average.
‡ Measured using the Bristol Stool Form Scale. Scores range from 1 (separate hard lumps) to 7 (liquid consistency).
§ Measured using the Ease of Evacuation Scale. Scores range from 1 (manual disimpaction) to 7 (incontinent).

explain the cognitive improvement observed in the RASMET and KARMET studies as well as the normalization in circadian rhythm and improvement in sleep noted in RASMET (18). We also found widespread inhibition of the visual cortex and, notably, of subcortical regions, such as lateral geniculate nuclei, superior colliculi, and interpeduncular and dorsal raphe nuclei; these structures are intimately implicated in the generation of visual hallucinations (Video and Supplement Figure 6). Inhibition of these structures may explain the improvement in hallucinations observed in both studies. Taken together, our preclinical studies predicted that orally administered ENT-01 should improve both bowel motility and gut–brain communication in persons with PD.

This study has several limitations. We have not validated the proposed cellular mechanism of action of ENT-01 in our patients. This would require electrophysiologic studies on intestinal tissue from patients with PD. However, our hypothesis also predicts that long-term administration of ENT-01 should reduce the accumulation of αS aggregates within the ENS. Studies that measure αS within enteric neurons in upper GI biopsies before and after long-term administration of ENT-01 would be required. The number of patients with psychosis or dementia was limited, and although similar responses were observed in the open-label study, these findings must be evaluated in future trials dedicated to PD psychosis and dementia. Finally, given the brief treatment period, the safety of ENT-01 will need to be evaluated for longer exposures in future studies.

In conclusion, we have shown that in patients with PD, targeting αS in the ENS leads to an improvement in bowel function and possibly neurologic symptoms. This study replicates the results of a previous open-label study. Future studies will focus on the effect of longer treatment periods on PD-related constipation and neurologic symptoms and on our understanding of the compound's mechanism of action in the human GI tract.

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Data Sharing Statement: The following data will be made available: complete deidentified patient data set. The following supporting documents will be made available: analytical/statistical code and informed consent form. These items will be made available to anyone, for any purpose, after approval (restrictions: none).

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