Protocol Title:

Treating Bacterial Overgrowth in Parkinson's Disease

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Amendment 3

HYPOTHESIS AND SPECIFIC AIMS

Parkinson's disease (PD) is a progressive neurodegenerative disorder that includes motor and non-motor impairment, including gastrointestinal (GI) dysfunction, one of the earliest abnormalities. The GI dysfunction is expressed as reduction of intestinal motility, which puts PD patients at risk for developing small intestinal bacterial overgrowth (SIBO), which develops in about 50% of PD patients, compared to only 8-20% of healthy age-matched controls[1, 2]. SIBO-positive PD patients have poorer response to therapy and more motor fluctuations than non-SIBO PD patients. Finally, open-label data suggest that the antibiotic rifaximin leads to eradication of SIBO and improvement in motor control in affected PD patients.

Here we propose to collect pilot data that would support the submission of a U01 application to the NIH for a large randomized controlled trial investigating the effect of SIBO eradication on reducing motor complications in PD patients. **Our central hypothesis is that SIBO-positive PD patients treated with rifaximin will exhibit an improvement in motor fluctuations as measured by a decrease in daily "off" time.**

Primary endpoints: to determine the change in "off" time after rifaximin treatment as measured by both patient report (the Hauser PD diary) and an objective monitoring system (Kinesia 360). "Off" time will be compared using a between-subjects analysis (rifaximin versus placebo), as well as a within-subjects analysis comparing each subject's baseline to 1 and 3 months post-rifaximin treatment. Examining 2 post-treatment time points will provide an estimate for the optimal time point for the primary outcome measure as well as for treatment duration in a future study.

Secondary endpoints:

1. **to investigate whether the treatment effect of rifaximin varies by method of SIBO detection.** We propose to use two non-invasive methods of SIBO detection, serum unconjugated bile acids versus a combination of lactulose and glucose breath tests. Thus, we can determine if there is a differential treatment effect based on method of detection, and use these data to select the detection method for a larger, randomized trial.
2. **To compare outcomes of the Hauser diary versus the Kinesia 360 monitoring system.** Within-patient results will be compared for sensitivity and effect size, and these data will be used to select the primary endpoint for a larger, randomized trial.

Tertiary endpoints:

1. Change in daily "on" time with no dyskinesia and without troublesome dyskinesia.
2. Change in daily "on" time with troublesome dyskinesia
3. Change in motor subscale (part III) of the Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS)
4. Change in the complication of therapy subscale (Part IV) scores of the MDS-UPDRS
5. Presence/recurrence of SIBO 1 month post-treatment
6. Correlation between the treatment effect of rifaxmin (change in "off" time) and SIBO eradication as measured by both non-invasive methods of SIBO detection.
7. Change in Patient Global Impression of Change (PGI-C).

The current proposal is designed to demonstrate our ability to detect and treat SIBO in PD patients with motor fluctuations, to estimate the effect size and standard deviation of motor improvements, and to inform selection of the best SIBO detection method. In addition, it will help us determine the optimal timeline for assessing motor endpoints, and estimate the duration of benefits after treatment.

BACKGROUND AND SIGNIFICANCE

Parkinson's disease and Motor Fluctuations: Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting 1-2% of the population over the age of 65 years [3]. It is a chronic, progressive disease characterized by degeneration of several neuronal cell types, including dopaminergic neurons of the substantia nigra pars compacta [4]. The symptomatic treatment for PD relies, in part, on the restoration of dopaminergic tone in the motor circuits of the brain, which is accomplished mainly through oral levodopa therapy, a precursor of dopamine. As PD progresses, many patients develop motor fluctuations – that is, alternating periods of "on" time, during which the patient enjoys a good response to medication, and "off" time, during which the patient experiences symptoms of their underlying parkinsonism, including impaired mobility [5]. Other motor fluctuations related to suboptimal therapy are episodes of no-on, delayed on (or prolonged time-to-on), predictable wearing off, and unpredictable on-off fluctuations, all of which collectively contribute to increasing a patient's total "off" time. In addition, many advanced PD patients experience dyskinesia (involuntary movements) as a side effect of levodopa during the peak of a dosing cycle. These motor complications are associated with the erratic pharmacokinetic profile of levodopa, which has been suggested to be related to delayed GI transit and SIBO (see below).

Small intestinal bacterial overgrowth: SIBO is a condition of increased bacterial density in the small intestine, and is defined as having greater than 10^5 colony forming units of colonic type bacterial flora per milliliter [6, 7]. SIBO is associated with various GI disorders including intestinal dysmotility, gastric achlorhydria, and anatomical abnormalities [8]. In such circumstances, bacteria that normally colonize the colon migrate proximally into the small bowel.

SIBO in PD: Recent studies have shown that SIBO develops in about 54% of PD patients with motor fluctuations, compared to only 8-20% of healthy age-matched controls [1, 2]. A third study found SIBO prevalence of only 25% in PD [9]; however there are important methodological differences between these studies. The latter study used only one diagnostic breath test, the lactulose breath test (LBT, described below) as opposed to LBT and glucose breath test (GBT), to detect SIBO, and secondly, their study population included PD patients *without* motor fluctuations. Thus, 54% is likely a more accurate estimate of SIBO prevalence in the study population proposed here. These recent studies report that PD patients with concurrent SIBO have poorer response to levodopa and more motor fluctuations than non-SIBO PD patients. Finally, they showed, using an unblinded design, that SIBO eradication using the antibiotic rifaximin leads to improved motor control in affected PD patients.

SIBO diagnosis and treatment: Currently, the "gold standard" for the diagnosis of SIBO is duodenal aspirate to quantify number of bacterial organisms. However, this method of diagnosis is invasive and expensive, rendering this diagnostic tool of limited clinical application. The alternative of greater use in clinical practice is the hydrogen breath tests LBT and GBT. These tests are based on the fact that the only source of hydrogen in the human body is from bacterial metabolism of carbohydrates. When ingested carbohydrates are exposed to intestinal bacteria, hydrogen is produced and expired. The most common carbohydrate substrates used for this test are glucose and lactulose. Glucose, which is readily absorbed in the proximal small bowel, in healthy conditions rarely reaches the colon or the hydrogen-producing bacteria found there. Thus, the GBT test is an attractive method for the detection of proximal SIBO [10, 11]. Lactulose is absorbed more distally, and the LBT is therefore a better indicator of distal SIBO. However, the sensitivity and specificity of the GBT has been reported to be only 62% and 83%, and for the LBT 68% and 44%, causing the clinical utility of these methods used in isolation to be questioned [12], and lending rationale to the combined use of these tests to increase the diagnostic yield.

A third, less commonly-used diagnostic tool for SIBO is the measurement of unconjugated bile acids (UBA), which relies on the physiology of bile acids and their relationship with bacteria. Bile acids are synthesized from cholesterol in the liver, where they are conjugated with glycine or taurine prior to secretion into the biliary tract and subsequently the intestinal lumen. Intestinal bacteria, mainly those normally found in the distal part of the gut, deconjugate a portion of those bile acids, and those UBA are absorbed and released into systemic circulation. Thus, the concentration and fraction of serum UBA are indicative of intestinal bacterial activity and can be used as a diagnostic tool for SIBO [13, 14].

Treatment of SIBO relies on antibiotics, with Rifamixin being the most common and well-validated for this indication. Treatment duration aimed at SIBO eradication is seven days, with a dose of 1600 mg/day

shown to be most effective, without affecting patient compliance or increasing incidence of side effects [15].

Significance: Our proposal gathers input from a multidisciplinary team of experts from neurology, endocrinology, and gastroendocrinology to evaluate a novel therapeutic option that has the potential to reduce motor fluctuations in more than half of all PD patients and improve functional outcomes. Thus, our proposal explores a heretofore untapped management strategy and may positively alter the standard of care in PD.

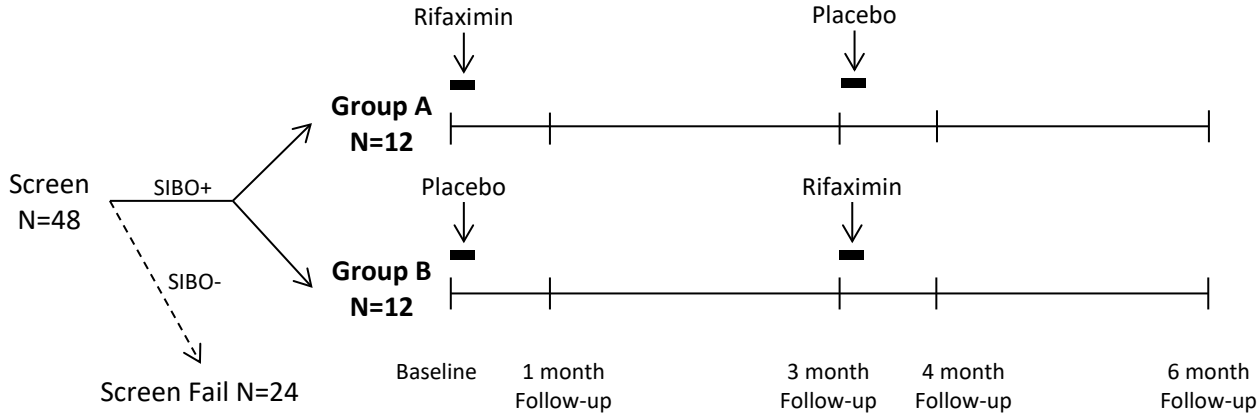
INVESTIGATOR EXPERIENCE

Dr. Alberto Espay is a licensed neurologist who has been conducting research in Parkinson's disease and other movement disorders for more than 10 years. He serves as the director of the James J and Joan A Gardner Center for Parkinson's disease and Movement Disorders at the University of Cincinnati, is staffed by five fellowship-trained movement disorders specialists and four dedicated full-time research coordinators. He oversees over 4,000 annual patient visits and directly manages over 1,245 patients with PD each year.

Dr. Hilary Wilson-Perez received her PhD in Neuroscience in 2012. She has experience in basic and clinical research in neuroendocrinology (diabetes and obesity), and has been working as a research coordinator in Movement Disorders since January 2014. Thus, she has the training and hands-on experience to handle the scientific and logistical aspects of the current study.

RESEARCH DESIGN AND METHODS

Study Plan: PD patients with motor fluctuations (≥4 hours of self-reported "off" time per day) will be screened for SIBO using breath tests (LBT and GBT). Subjects testing positive by either method will be eligible for enrollment, whereas those testing negative by *both* methods will be considered screen failures, and will be discontinued from the study. We expect that approximately 50% of screened patients will be SIBO-positive [1, 2]; therefore we plan to screen 48 subjects to achieve an enrollment of 24. SIBO-positive subjects will be asked to return for a baseline visit 2-4 weeks after screening, and to complete the standardized Hauser diary[16, 17] capturing daily "on" and "off" time for 3 days prior to the baseline visit. Daily "off" time must average at least 4 hours per day to proceed with enrollment. They will also use the Kinesia 360 monitoring system during this time.



Subjects meeting all inclusion/exclusion criteria will be enrolled and randomly assigned to one of two groups in a randomized, double-blind, crossover fashion (see Figure), each treated with a one-week course of the antibiotic rifaximin as well as a one-week course of matching placebo. Group A will receive rifaximin at baseline and placebo at 3 months, whereas group B will receive placebo at baseline and

rifaximin at 3 months. Outcome measures including motor control will be assessed at the following time points: baseline (BL, pre-treatment), 1 month, 3 months, 4 months, and 6 months.

Clinical assessments will include a motor examination and evaluation of motor complications using sections III and IV of the MDS-UPDRS, respectively. Patients will be trained in the correct use of the PD Hauser diary to log "on" and "off" time, as described below.

Blinding: Amendment 3 modifies this study to be a fully double-blind crossover, such that neither participants nor researchers will know when the subject receives rifaximin or placebo. This will be explained to the participants, so they will know that they will receive one treatment course of each during the study, but they will not know in which order they were treated. The results of the breath tests (SIBO-positive or SIBO-negative) will only be revealed to the subject at screening, which determines their ability to enroll in the study. The results of subsequent breath tests on enrolled subjects will not be revealed to subjects or the motor assessor to avoid bias on symptomatic outcome measures.

Breath Tests: GBT and LBT will be performed according to standard procedures on separate days. Subjects are placed on a restricted, low-carbohydrate diet the day before the test, and fast for 12 hours prior to GBT and LBT. A baseline alveolar air sample will be collected before ingestion of glucose (1g/kg of body weight) or lactulose (10g), followed by sampling every 20 minutes for 3 hours. The diagnosis of SIBO is established by expert review by an experienced Gastroenterologist. Generally, a positive GBT is defined as an increase in exhaled hydrogen ≥ 10 parts per million above baseline. A positive LBT is characterized by two distinguishable hydrogen peaks (SIBO and colonic peaks) after lactulose ingestion [19]. However, individual variances in hydrogen dynamics require expert review for a final determination.

- Screening breath tests (LBT and GBT) that do not exhibit a rise in hydrogen levels of at least 8 ppm in the first 80 minutes will be discontinued and considered negative. All others will be completed for later determination.
- After screening, all enrolled subjects will have breath tests performed to completion to avoid revealing the results of the test to the participant.

Measurement of Serum Unconjugated Bile Acids: Serum will be obtained from subjects in order to measure concentration and fractionation of UBA. Batched samples will be analyzed in a post-hoc fashion, and not used for screening (study inclusion) purposes. The major primary and secondary unconjugated and conjugated bile acids will be measured in serum using a routine validated LC-MS/MS method according to CCHMC SOP # PATH.CMS.1033.

Study Drug and Placebo: Rifaximin treatment will be a 7-day course of 550mg three times per day (1650mg/day). The drug is supplied as 550mg tablets. Matching placebo tablets will be administered in the same fashion.

PD Diary: Patients will be instructed to use the Hauser diary [16] to log their motor fluctuations for three consecutive days prior to each study visit (concurrent with Kinesia monitoring). Each diary page is a grid representing a 24-hour day in which patients mark each half-hour period as one of 5 categories: Asleep, OFF, ON without dyskinesia, ON with non-troublesome dyskinesia, or ON with troublesome dyskinesia.

Kinesia 360 Monitoring. Kinesia is an FDA cleared to market device for objectively assessing Parkinson's disease symptom severity. The system includes wireless motion sensors and a tablet PC. Subjects will be trained in its use at screening, and will use it for 3 consecutive days prior to each subsequent study visit (concurrent with PD diary completion).

MDS-UPDRS On/off evaluation: The MDS-UPDRS part III (motor examination) will be used to evaluate subjects in both the "on" and "off" conditions. MDS-UPDRS part IV (motor complications) is an interview-based assessment that refers to the subject's functioning over the past week, and thus can be conducted in either the "on" or "off" state. These will be completed at all study visits except screening. The "off"

portion will be conducted after subjects have abstained from PD medications for 12 hours. Following motor evaluation, they will take their normal dose of PD medication(s), and will be evaluated again one hour later, or when the subject reports feeling fully "on".

Patient Global Impression of Change. The subject will rate his/her impression of change in PD symptoms from baseline (i.e., just before subject's most recent study drug treatment) to the time of assessment. The PGI-C is assessed on a 7-point scale ranging from "very much improved" to "very much worse".

Screen Failures and Rescreening:

Subjects whose breath tests are negative will be considered screen failures and will not be enrolled in the study. However, bacterial populations in the intestine may change with diet, antibiotic treatment, or other conditions. For these reasons, the investigator and subject may elect re-screening following negative results. Elective rescreening following two negative breath tests must be at least 14 days after previous breath testing.

In other cases, breath test results may be borderline, incomplete, or otherwise undetermined as deemed by expert review. These subjects may return for rescreening 1-60 days following previous breath testing. At minimum, the previously-undetermined breath test (GBT or LBT) must be repeated. Both breath tests may be repeated at investigator discretion. If more than 60 days have passed following breath testing, then both GBT and LBT must be completed.

Randomization, Sample Size Estimation and Statistical Analysis Plan: PD patients testing positive for SIBO will be randomized to group A or B in equal numbers using a randomization code generated by the statistician.

Our primary outcome, change in "off" time, will be analyzed using two separate analyses. A within-subjects comparison (paired t-test) will compare all subjects (n=24) before and after rifaximin treatment, whereas a between-subjects analysis (two-sample t-test) will compare placebo treatment (n=12) to rifaximin treatment (n=12). Therefore, we will have one analysis with greater power to detect a treatment effect, and a second analysis with greater validity for designing a future placebo-controlled trial.

Based on previous data, we have estimated that SIBO-positive PD patients have 5.1 hours of "off" time, with a treatment effect of rifaximin to decrease "off" time by 2 hours. Standard deviation is estimated to be approximately 1.5 to 2 hours [2].

Because all subjects will ultimately receive rifaximin treatment, a within-subjects analysis will compare all 24 subjects before and (1 and 3 months) after treatment using paired t-tests. For group A, this corresponds to baseline versus 1 month, and baseline versus 3 months. For group B, this corresponds to 3 months (end of placebo treatment, and baseline for rifaximin treatment) versus 4 months, and 3 months versus 6 months. Assuming a mean "off" time before treatment of 5.1 hours, a conservative estimate of standard deviation of 2.5 hours, and a moderate correlation of 0.7 between the measurements before and after treatment, we will have 99.8% power to detect a difference of 2 hours, and 95.3% power to detect a difference of 1.5 hours of "off" time.

A second analysis will compare placebo treatment versus rifaximin treatment between subjects. We will use a two-sample t-test to compare the "off" time between groups A and B at 1 month and 3 months past baseline. Assuming initial "off" time in both groups to be 5.1 hours, a decrease in "off" time of 2 hours in the rifaximin group, and no change in the placebo group, we will have 87.7% power or 64.9% power to detect the difference between groups based on standard deviations of 1.5 and 2.0 hours, respectively. Due to the nature of this pilot study, and the imprecise estimates of standard deviation, it is possible that this analysis will not have the power to detect a treatment difference between groups. However, these data will provide valuable estimates of mean and standard deviation which can be used

to design a larger clinical trial. Furthermore, the within-subjects analysis described above should have ample power to detect a treatment effect.

We expect some people to be identified as having SIBO using serum unconjugated bile acids only, some to be identified using breath tests only, and others to test positive by both methods. To determine whether treatment effect varies by detection method, we will compare the change in "off" time at one month after the start of treatment in the three groups using a one-way ANOVA.

All power estimations were made using PROC POWER in SAS 9.3. The generation of codes for randomization and all the analyses will also be conducted using SAS 9.3.

Treatment of missing data will be handled on a case-by-case basis. The pilot nature and modest sample size of this study makes imputation not appropriate. The decision to exclude a single missing value, series of longitudinal values for a patient, or a patient's entire data set will vary depending on time of drop-out (if applicable) and type of missing data.

Strategies to mitigate potential pitfalls: To avoid normal day-to-day PD-associated variability to substantially alter the "off" and "on" diary outcomes, we will conduct all measurements at the same time of day in each subject. Also, we do anticipate a number of early drop-outs due to inability to comply with the extensive set of study-related procedures. This might pre-select more motivated patients and bias recruitment in favor of those willing and able to invest the time and effort. However, it is likely these subjects will have an "off" time substantially above that of the inclusion cutoff, thus increasing the range of effect by rifaximin. Finally, SIBO measurements have not been previously applied to PD patients. We will closely monitor for quality metrics and patient adherence given the unique features of the PD population compared to patients with primary GI disorders.

Next steps: Data gathered in this study will inform the design of a large, definitive NIH-sponsored study on the value of rifaximin as a promising management strategy in the care of patients with PD.

Data Storage and Confidentiality: All records from this study will be maintained in a secure setting. Electronic records will be stored on a password protected computer. Records will not have any patient health information (PHI) associated with them. These records will be kept indefinitely to allow for re-analysis of data, if needed. The data will be verified by multiple members of the research team, including the PI. The data will never be accessible without a secured password.

Setting: Study visits will take place at the Medical Arts Building Neurology Suite 3200.

HUMAN SUBJECTS

Consecutive consenting patients will be recruited from the University of Cincinnati Movement Disorders Center. We expect to screen 4-5 patients per month, and to complete enrollment in one year (48 screened, 24 enrolled). Assessments will be ongoing for an additional 6 months.

Inclusion criteria:

1. Diagnosis of PD according to the UK Brain Bank criteria[18]
2. Daily "off" time ≥4 hours according to subject-completed Hauser diary.
3. No changes in levodopa or any other dopaminergic medications will be expected during the course of the study, at the judgment of the treating clinician.
4. Montreal Cognitive Assessment (MoCA) ≥ 24.
5. Age 18 and older.

Exclusion criteria:

1. Any comorbid non-PD-associated gastrointestinal (e.g., achlorhydria) or systemic disease that may alter absorption or confound the study results, including immune disease.

2. Concurrent exposure to immunosuppressive drugs or antibiotic treatment, or within 30 days of screening.
3. Known allergy to rifaximin
4. Women who are pregnant, lactating, or plan to become pregnant.

Risk/Benefits: This study involves more than minimal risk due to use of study drug and the breath tests (which involve ingestion of sugar). The risks associated with Rifaximin treatment are similar to placebo, and include flatulence, headache, abdominal pain, rectal tenesmus, defecation urgency, nausea, constipation, pyrexia, and vomiting. The risks associated with breath testing are related to the possibility of poor digestion and absorption of carbohydrates, and may include bloating, distention, pain, diarrhea, and hypoglycemia. These risks are similar to what participants would experience any time they would take an antibiotic or ingest a moderate amount of carbohydrate.

Withholding PD medications for the MDS-UPDRS On/off evaluation may cause a temporary worsening in PD symptoms including tremor, bradykinesia, rigidity, freezing, and other PD-associated symptoms.

There are no controlled data regarding the use of Rifaximin during pregnancy or lactation, and it is not known whether Rifaximin is excreted in breastmilk. Subjects may not participate if they are pregnant, lactating, or plan to become pregnant or cause a pregnancy. Subjects will be counseled on birth control methods.

Risks will be managed by inquiring subjects at each visit of any new symptoms, and encouraging them to contact the study team with any new symptoms or concerns. Adverse events will be reported according to the reporting requirements of UC Human Research Protection Institutional Policy II.02 *Reporting Unanticipated Problems.* Participants will be promptly discontinued from study medication or study procedures if the investigator believes it is in his/her best interest.

This research may benefit the subjects directly by eradicating SIBO, which may in turn improve motor symptoms. Furthermore, this study has the potential to impact more than half of all Parkinson's patients to positively improve their PD symptoms.

Payment: Subjects will not be paid for their participation in this study.

Subject Costs: There will be no costs to the research participants. Study drug, procedures, and assessments will be provided at no cost.

Consent: See separate document.

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