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2 Introduction

2.1 Preface

Falls and other abnormalities of gait and balance are common in Parkinson's disease (PD), are largely levodopa unresponsive, and are major contributors to morbidity and mortality. The central theme of the University of Michigan Udall Center is that degeneration of basal forebrain (BF) and pedunculopontine (PPN) cholinergic projections, in the context of degraded striatal motor control, contributes significantly to PD gait and balance deficits. Our preclinical work identifies attentional deficits, secondary to loss of cortical cholinergic afferents, as a key mechanism through which cholinergic deficits contribute to impaired gait and balance. The goal of this pilot target engagement/pharmacodynamic clinical study is to assess α4β2* nicotinic cholinergic receptors (nAChRs) as a therapeutic target for improving gait, balance, and attentional capacity in PD.

To demonstrate the $\alpha4\beta2^*$ nAChRs are appropriate therapeutic targets inPD, it is necessary to study key pharmacokinetic-pharmacodynamic features of $\alpha4\beta2^*$ nAchR in the context of the degenerating, hypocholinergic PD brain, a pathologic environment in which they may exhibit unique features. This personalized medicine approach focuses our studies on hypocholinergic PD subjects. We will assess $\alpha4\beta2^*$ nAChR features using Positon Emission Tomography (PET) imaging with the $\alpha4\beta2^*$ nAChR ligand [^{18}F]flubatine, subacute administration of the $\alpha4\beta2^*$ nAChR partial agonist varenicline (VCN), and laboratory measures of gait, balance, and attention. We will perform a pharmacodynamics study with subacute VCN administration to determine if $\alpha4\beta2^*$ nAChR stimulation improves laboratory measures of gait function, postural control, and attentional function in hypocholinergic PD subjects.

2.2 Scope of the analyses

These analyses will assess the efficacy and safety of varenicline in comparison with placebo, addressing the primary and secondary objectives of the study.

3 Study Objectives and End points

3.1 Study Objectives

Primary Objective

- To assess the effects of pharmacologic α4β2* nAChR activation on laboratory measures of gait function and postural control in PD subjects with cholinergic deficits.

Secondary Objectives

- To assess the effects of pharmacologic α4β2* nAChR activation on a measure of attentional function (SAT; sustained attention test) in PD subjects with cholinergic deficits.

Exploratory Objectives

- To assess the effects of pharmacologic α4β2* nAChR activation on additional measures of motor, cognition, and behavior in PD subjects with cholinergic deficits.

3.2 End points

All efficacy end points are assessed at the end of each treatment period (days 22 and 64).

3.2.1 Primary Efficacy End points

- JERK (postural measure based on time-based derivative of lower trunk accelerations)
- Gait speed under normal pace and no dual task conditions

3.2.2 Secondary Efficacy End points

- Sustained attention test (SAT)

3.2.3 **Exploratory Efficacy End points**

- Movement Disorder Society Unified Parkinson's Disease Rating Scale, part III (MD-UPDRSIII; "on" state)
    - Total score
    - Postural instability and gait disorder (PIGD) subscore
- Gait measures
    - Gait speed under fast pace and no dual task conditions
    - Gait speed under normal pace and dual task conditions minus gait speed under normal pace and no dual task conditions
    - Gait speed under fast pace and dual task conditions minus gait speed under fast pace and no dual task conditions
    - Cadence under normal pace and no dual task conditions
    - Cadence under fast pace and no dual task conditions
    - Mean stride length under normal pace and no dual task conditions
    - Mean stride length under fast pace and no dual task conditions
    - % coefficient of variation (CV) stride length under normal pace and no dual task conditions
    - %CV stride length under fast pace and no dual task conditions
    - Mean stride time under normal pace and no dual task conditions
    - Mean stride time under fast pace and no dual task conditions
    - %CV stride time under normal pace and no dual task conditions
    - %CV stride time under fast pace and no dual task conditions
    - Mean stride width under normal pace and no dual task conditions
    - Mean stride width under fast pace and no dual task conditions
    - %CV stride width under normal pace and no dual task conditions
    - %CV stride width under fast pace and no dual task conditions
- Postural measures
    - Mean sway velocity
    - Root mean square sway distance (Sway RMS)
    - Timed Up and Go (iTUG)
- Cognitive measures
    - Montreal Cognitive Assessment (MoCA) score
    - Wechsler Adult Intelligence Scale (WAIS-III) Digit Symbol score
    - California Verbal Learning Test (CVLT) Short Term Memory score
    - CVLT Long Term Memory score
    - CVLT Recognition score
    - Delis-Kaplan Executive Function System (D-KEFS) Stroop III (interference) score
    - D-KEFS Sorting total score
    - D-KEFS Verbal Fluency – Letters total score
    - D-KEFS Verbal Fluency – Animal score
    - Judgment of Line Orientation (JOLO) score
    - Trail Making Test (TMT) 4 score
    - Distractor Sustained Attention Test (dSAT) score
- Behavioral measures
    - Geriatric Depression Scale (GDS)
    - Questionnaire for Impulsive-Compulsive Disorders in PD (QUIPs)

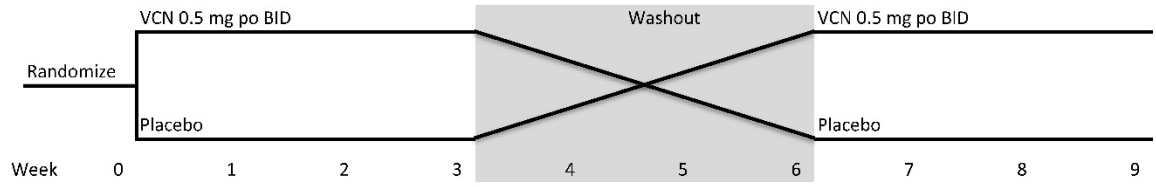
4 Study Methods

4.1 General Study Design and Plan

This study is a single-center, randomized placebo-controlled double-blind crossover study of VCN in participants with PD to assess its effects on measures of gait, balance, and cognition. Participants were randomized 1:1 to one of two treatment sequences: placebo followed by VCN 0.5 mg b.i.d., or

VCN followed by placebo. Participants received an initial 0.25 mg dose or equivalent placebo following baseline evaluations and were monitored for 4 hours after initial study medication administration with total daily dose or equivalent placebo escalated over the next 2 days. Treatment periods were 3 weeks in duration and interrupted by a 3-week washout period. Participants underwent a standard evaluation at baseline, at the end of the first treatment period (week 3 [day 22]), at end of the washout period-beginning of the second treatment period (week 6 [day43]), and at the end of the second treatment period (week 9 [day 64]).

The schema below describes the main elements of the study design:



4.2 Inclusion-Exclusion Criteria and General Study Population

4.2.1 Inclusion Criteria

1. PD diagnosis will be based on the United Kingdom Parkinson's Disease Society Brain Bank Research Center (UKPDSBRC) clinical diagnostic criteria. We will enrich the cohort by recruiting subjects at modified Hoehn and Yahr stages 2 or higher, duration of motor disease 5 years or longer, age >65 years, or the PIGD phenotype. Duration of motor disease will be defined as the time between onset of motor symptoms and time of entry into the study. The PIGD phenotype is defined as described previously. PD subjects with defined cholinergic deficits will be recruited as described in Project II. PD subjects will have cortical cholinergic deficits based on 5th percentile cutoff of the normal controls as defined previously.
2. Stable dopaminergic replacement therapy for 3 months prior to enrollment and expected to maintain stable dopaminergic therapy for duration of study participation.

4.2.2 Exclusion Criteria

1. Other disorders which may resemble PD with or without dementia, such as vascular dementia, normal pressure hydrocephalus, progressive supranuclear palsy, multiple system atrophy, corticobasal ganglionic degeneration, or toxic causes of parkinsonism. Prototypical cases have distinctive clinical profiles, like vertical supranuclear gaze palsy, early and severe dysautonomia or appendicular apraxia, which may differentiate them from idiopathic PD. The use of the UKPDSBRC clinical diagnostic criteria for PD will mitigate the inclusion of subjects with atypical parkinsonism and all participants will undergo [^{11}C]DTBZ PET to confirm striatal dopaminergic denervation.
2. Subjects on neuroleptic, anticholinergic (trihexiphenidyl, benztropine), or cholinesterase inhibitor drugs.
3. Current or previous (within last 6 months) use of any product or medication containing nicotinic agents, including use of tobacco products such as cigarettes, cigars, pipes, chewing tobacco, etc., e- cigarettes, OTC nicotine patches, chewing gum containing nicotine, or varenicline.
4. Evidence of a stroke or mass lesion on structural brain imaging (MRI).
5. Participants in whom magnetic resonance imaging (MRI) is contraindicated including, but not limited to, those with a pacemaker, presence of metallic fragments near the eyes or spinal cord, or cochlear implant.
6. Severe claustrophobia precluding MR or PET imaging
7. Subjects limited by participation in research procedures involving ionizing radiation.
8. Pregnancy (test within 48 hours of each PET session) or breastfeeding.
9. Significant risk of cardiovascular event.

10. Active, significant mood disorder.
11. History of seizures.
12. Active alcohol abuse.

4.3 Randomization and Blinding

Participants were randomized 1:1 to one of two treatment sequences: placebo followed by VCN 0.5 mg b.i.d., or VCN followed by placebo. A statistician prepared the randomization list using permuted blocks with random block sizes. The list with randomization number and treatment allocation was sent to the research pharmacy and a blinded list of randomization numbers was sent to the study coordinator. After patient consent was completed and eligibility confirmed, the coordinator assigned the next randomization number to the participant, and sent a prescription with participant ID and randomization number to the research pharmacist who dispensed the appropriate study medication. To mask drug, VCN pills or placebo were encapsulated in gelatin sheaths.

4.4 Study Assessments

4.4.1 Primary Efficacy Assessments

Two co-primary end points were selected: a measure of postural stability and gait function. JERK is the time-based derivative of lower trunk accelerations during standing spontaneous sway. JERK was chosen because it tracks postural instability in PD [1]. Normal pace gait speed with no distractors was chosen as prior studies indicated that neocortical cholinergic denervation is associated with slower gait speed in PD [2]. We hypothesized that VCN treated participants would ambulate faster and that VCN treatment would reduce JERK.

Postural stability was assessed with the Ambulatory Parkinson's Disease Monitoring (APDM) wearable sensor system (APDM Wearable Technologies, Inc.) using the iSWAY protocol, with participants standing on a foam pad with eyes open and eyes closed. Standard postural measures, including JERK, were assessed and calculated using the manufacturer's software (Mobility Lab Version 1). Gait analysis was performed on an 8-meter GAITRite pressure sensitive walkway (CIR Systems, Inc.) and standard parameters were analyzed using ProtoKinetics Movement Analysis Software (GAITRite version 5.09C; ProtoKinetics, LLC). Gait assessments were repeated with a dual-task protocol in which participants counted backwards by three starting at a random number (under 100) provided by the examiner.

4.4.2 Secondary Efficacy Assessments

We assessed attentional function with a Sustained Attention Test (SAT), established to reflect CNS cholinergic systems function in humans [3, 4, 5]. The SAT is performed with 2 conditions: without and with a distractor (dSAT). SAT and dSAT results are reported as the vigilance index, a measure that corrects estimates of accurate detection with penalties for false detections and not confounded by errors of omission [6]. SAT was selected as the second end point.

4.4.3 Exploratory Efficacy Assessments

Additional assessments of postural control, gait function and attentional function were measured as exploratory end points. Cognitive assessments were also measured for exploratory analyses.

Motor Assessments: Movement Disorder Society Unified Parkinson's Disease Rating Scale, part III (MD-UPDRSIII; "on" state); MDS-UPDRSIII postural instability and gait subscore (PIGD) subscale score (sum of items 3.1, 3.9-3.13); Gait Speed (normal pace); Gait Speed (fast pace); Gait Speed (normal pace - dual task); Gait Speed (fast pace – dual task); Postural stability measures – mean sway velocity, JERK, root mean square sway distance (RMS). To assess the effects of attentional loading, normal pace and fast pace gait were performed under dual task conditions. Dual task conditions typically lead to slower gait speed. Differences in gait speed between dual task and no dual task conditions are a measure of the attentional burden imposed by the dual task. To assess the effects of VCN on this aspect of gait performance, we compared the differences between no dual task and dual task gait speed between VCN and placebo treatment periods. We used the APDM system's iTUG

(Timed Up and Go) protocol to collect additional exploratory data.

Cognitive Assessments: For cognition, we used a general cognitive measure, the MoCA and selected tests to examine major cognitive domains but focusing on attention and executive function. MoCA; Wechsler Adult Intelligence Scale-III Digit Symbol modalities test; CVLT short term memory test; CVLT long term memory test; CVLT recognition test; D-KEFS Stroop III; D-KEFS sorting total; D-KEFS verbal fluency letters total; D-KEFS verbal fluency animals; D-KEFS Trail Making Test 4; JOLO test.

Behavioral Assessments: GDS

5 Sample Size

The sample size (planned initially at four participants per dosing group) for the VCN- α4β2* nAChR occupancy study was based on logistical considerations. For the Crossover study, we calculated that 33 participants would provide at least 80% power to detect within-patient treatment differences of 0.122 m/s in gait speed and -0.131 m2/s5 for JERK, assuming within-participant correlation of > 0.64 and > 0.72, respectively, using a paired t-test and a two-sided Type I error of 0.025 (Bonferoni adjustment for co-primary endpoints). This approach is conservative given our analysis method uses mixed effects models. Estimates for treatment differences were based on Bohnen et al. [2] for normal pace gait speed and Mancini et al. for JERK [7].

6 General Analysis Considerations

6.1 Timing of Analyses

The final analysis will be performed after all randomized participants have completed their 9-week visit or dropped out prior to their 9-week visit, all corresponding data have been entered, cleaned, locked and unblinded as per the data coordinating center's standard operating procedures. This statistical analysis plan document was finalized prior to the database lock and unblinding.

6.2 Analysis Populations

All randomized participants will be used in the analyses of subject disposition.

6.2.1 Intention to Treat Population

The main analysis set for efficacy is the intention to treat (ITT) population, defined as all participants randomized.

6.2.2 Compliant Population

A compliant population is used for sensitivity analyses of the efficacy end points. It is defined as the ITT population, excluding all participants who have major protocol deviations. Major protocol deviations are defined as eligibility criteria violations for which no exemption was granted, and without evidence of significant increases in VCN plasma levels between placebo and VCN treatment periods.

6.2.3 Safety Population

The Safety Population is defined as all participants who are randomized and receive at least one dose of study drug. The Safety Population will be used for all safety analyses, as well as demographic and baseline analyses.

6.3 Covariates and Subgroups

There are a limited number of covariates that will be incorporated in statistical models in our analyses because of the relatively small sample size in each treatment group: the baseline outcome measure. We will not impute missing values for baseline covariates.

6.4 Missing Data

We will summarize the extent of missing data over time for the primary end points. We will

investigate the missing data mechanism (missing at random, not missing at random), which is important for the validity of our analytic approaches, through exploratory analysis. Exploratory analyses will include plots of the mean profile of end points at weeks 0, 3, 6, and 9 by treatment sequence for those who have complete data throughout the study and those who don't.

The primary analysis of the primary end points assumes a missing at random mechanism. If data are not missing at random, we will use a multiple imputation approach within the pattern-mixture model framework [8]. The imputation models, applied sequentially for each missing data pattern, will include baseline value, treatment group, and demographic variables (age and gender), allowing for the dependence of later time points on earlier time points. If there are issues with over-fitting, the demographic variables will be omitted. The analysis model will include the same covariates used in the primary analysis of the primary end points and will incorporate the uncertainty due to imputation in the calculation of the standard error, as described by Rubin [9].

6.5 Interim Analyses and Data Monitoring

No formal interim analyses were planned nor carried out for this study. The study was overseen by a Data and Safety Monitoring Board (DSMB) that reviewed the pooled and by-treatment subject disposition, study conduct and safety data approximately every 6 months.

6.6 Multiple Testing

Two-sided p-values will be reported. For the co-primary endpoints, a Bonferonni adjustment for multiplicity will be applied. For all other endpoints, no adjustments for multiplicity will be made. Thus, p-values for secondary and exploratory outcomes will be interpreted with caution (note that this is a departure from the protocol). Means and standard errors will be provided to summarize treatment differences for efficacy end points.

7 Summary of Study Data

Descriptive summary statistics will be derived for all data at baseline, separately by treatment sequence and overall. For efficacy, exploratory and safety data, data will be presented by treatment group. Treatment group will be characterized as "Varenicline" and "Placebo"; for pooled summaries, "Overall" will be used as the column heading. All tables will be annotated with the total population size relevant to that table/treatment, including any missing observations.

For continuous variables, mean, standard deviation, median, interquartile range, minimum and maximum will be reported. For categorical variables, number and percentages will be reported (excluding missing values). Graphical methods will be heavily used in this pilot study to assess the pattern of response over time for key variables and to assess the relationships among variables.

7.1 Subject Disposition

The number of participants approached for study participation, the number consented and the number who did not consent (including reasons: screen failures, refusals) will be summarized in a CONSORT diagram. The number of participants who dropped out prior to randomization, and the reasons for dropout, will be summarized. The number randomized and treated and the number who dropped out by weeks 3, 6, and 9 will be provided, as well as the number in each of the analysis populations (i.e., ITT, Compliant, Safety). Reasons for post-treatment dropout will be provided.

7.2 Protocol Deviations

Major protocol deviations that exclude a patient from the Compliant Per Protocol Population are described in section 8.3.2. A listing of protocol deviations that exclude participants from the Compliant Population will be provided. A listing of participants who receive exemptions for study eligibility will also be provided.

7.3 Demographic and Baseline Variables

Demographic variables include: age at consent (defined as a continuous variable, e.g., 52.6 years), sex, race and ethnicity.

Baseline is defined as pre-treatment measures. Baseline variables include:

- Age at diagnosis (years)
- MDS-UPDRS III total score
- GDS score
- MoCA score

7.4 Treatment Compliance

A listing of compliance with study medication (# of doses taken by day) was provided by treatment and period.

8 Efficacy Analyses

8.1 Primary, Secondary and Exploratory Efficacy Analyses

For the Crossover study, linear mixed models containing treatment sequence, treatment period, treatment group, and dependent-variable baseline value, with participant within treatment sequence as a random effect, will be used for analysis of continuous outcomes. To compare differences between VCN and placebo, a test for carryover based on the sequence effect will be conducted using patient with sequence as the error term. Results will be presented as least squares (LS) mean and standard error (SE). The co-primary endpoints will be tested at the 2-sided, 0.025, significance level. All other tests will be based on a 2-sided significance level of 0.05; no adjustments will be made for additional multiple comparisons. Primary, secondary and key efficacy endpoints will be analyzed in all randomized participants (intention-to-treat [ITT] population) and in the Compliant Population.

Additional sensitivity analyses will be performed for UPDSR-III total and PIG subscale scores, where baseline degree of total cortical cholinergic denervation (from PET scans) and baseline degree of striatal dopamine denervation (from PET scans) were incorporated as covariates in separate models. These models will also be run for gait speed end points.

9 Safety Analyses

Safety endpoints will be analyzed in all randomized participants who received at least one dose of study medication (Safety Population). We will include adverse events that occurred in the washout period with the treatment given in period one.

Safety data, including adverse events (AEs) and the Columbia-Suicide Severity Rating Scale (C-SSRS), will be summarized descriptively by treatment group for the Safety Population.

9.1 Adverse Events

Treatment-emergent adverse events are AEs that start on or after the first study day treatment is administered. The causal relationship of the AE to the study drug is determined by the site investigator as “not related” or “related”. Adverse event severity grades are reported according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. If the CTCAE does not have a grading for a particular adverse event, the severity of the event is reported by the investigator as mild, moderate, severe, or very severe. In the case of multiple occurrences of the same AE within the same subject, AEs will be summarized according to the maximum severity reported for each body system and overall.

Descriptive summary statistics for treatment-emergent AEs will be reported. The number of treatment-emergent AEs and the frequencies (number and percentage) of participants with one or more treatment-emergent AE will be summarized by treatment group, overall, by severity, and by body system. Coding of adverse events into body system was performed by the study chair for adverse events and by the medical monitors for serious adverse events. All treatment-emergent AEs related to study drug will be summarized, as will the frequencies of participants with one or more treatment-emergent AE related to study drug. Similarly, all treatment-emergent AEs causing study

discontinuation, and frequencies of participants experience these, will be summarized.

A subject listing of all treatment-emergent AEs and treatment-emergent AEs causing study discontinuation will be presented.

In accordance with clincaltrial.gov reporting requirements, the following table summarizing adverse events is required and will be provided:

- Other (Not Including Serious) Adverse Events: A table of anticipated and unanticipated events (not included in the serious adverse event table) that exceed 5% within either treatment group, grouped by organ system, with number and frequency of such events in each treatment group.

AEs that occurred after consent and before treatment will be listed.

The summary statistics will be produced in accordance with section 9.

9.2 Deaths, Serious Adverse Events and other Significant Adverse Events

Descriptive summary statistics for treatment-emergent serious adverse events (SAEs) will be reported. The number of treatment-emergent SAEs and the frequencies (number and percentage) of participants with one or more treatment-emergent SAE will be summarized by treatment group, overall and by body system. Coding into body system was performed by the data coordinating center for SAEs. All treatment-emergent SAEs related to study drug will be summarized, as will the frequencies of participants with one or more treatment-emergent SAE related to study drug. Similarly, all treatment-emergent SAEs causing study discontinuation, and frequency of participants experiencing these, will be summarized.

A subject listing of all treatment emergent SAEs, SAEs causing study discontinuation, and deaths will be presented.

In accordance with clinicaltrials.gov reporting requirements, the tables below summarizing deaths and SAEs are required:

- All-Cause Mortality: A table of all anticipated and unanticipated deaths due to any cause, with number and frequency of such events in each treatment group.
- Serious Adverse Events: A table of all anticipated and unanticipated serious adverse events, grouped by organ system, with number and frequency of such events in each treatment group.

The summary statistics will be produced in accordance with section 9.

9.3 Other Safety Measures

Listing of C-SSRS significant suicidal ideation (suicidal ideation >2) will be provided.

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