TRIAL FULL TITLE	Cholinergic Mechanisms of Gait Dysfunction in Parkinson's Disease – Proj#3
SAP VERSION	1.0
SAP VERSION DATE	01AUG2019
TRIAL STATISTICIAN	Cathie Spino, DSc
Protocol Version (SAP associated with)	Protocol Version 9.0 April 17, 2020
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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.

3 Study Objectives and End points

3.1 Study Objectives

Primary Objective

- To use [18F]flubatine PET to assess varenicline (VCN) occupancy of brain α4β2* nAChRs after subacute VCN administration to the subgroup of PD subjects with cholinergic deficits in comparison with normal control subjects.

3.2 End points

All efficacy end points are assessed at the end of treatment (day 10) and at follow-up day 15.

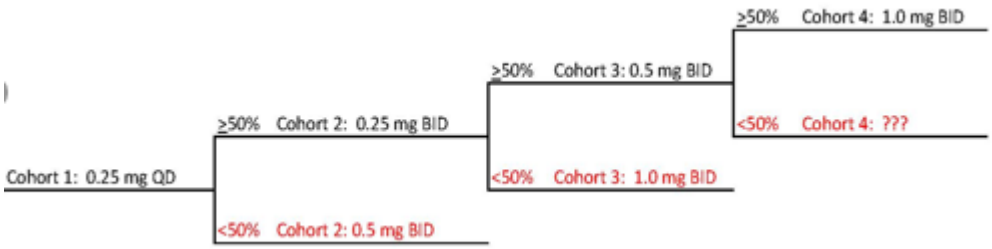
3.2.1 Primary Efficacy End points

VCN displacement of thalamic [^{18}F]Flubatine binding to α4β2* nAChRs (the region with the highest [^{18}F]Flubatine binding). Receptor occupancy is defined as [(Day 10 – Day 15) / Day 15] x 100%.

4 Study Methods

4.1 General Study Design and Plan

The goal of the first experiment was to select the lowest dose of VCN to produce high (90%) receptor occupancy. α4β2* nAChR receptor occupancy is defined as the difference between [^{18}F]Flubatine binding before and VCN discontinuation, i.e., 10 days after initiation of VCN administration (Day 10) and 5 days after VCN discontinuation (Day 15).



The goal is to have 3/3 subjects achieve >90% receptor occupancy. The dose schedule will be increment, as shown in the figure above, until there is no additional reduction in [18F]Flubatine binding, i.e., remaining binding is non-specific binding, and the penultimate maximum dose will define the dose to be used in the future crossover study.

The second experiment replicates the first experiment in a healthy volunteer population, matched for age. This experiment occurred after the results of the first experiment were known. Thus, only 3 dose groups were assess: 0.25 mg QD, 0.25 mg BID and 0.5 mg BID.

Participants received their first dose of varenicline on Day 1, followed by dose escalations on days 2 and 3 (as needed). On Day 10, they discontinued varenicline and received their first flubatine PET scale. Five days after discontinuation of varenicline (Day 15), they received their second flubatine PET scan.

4.2 Inclusion-Exclusion Criteria for Experiment 1 (PD patients with cholinergic deficits)

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 Inclusion-Exclusion Criteria for Experiment 2 (Healthy Controls)

Normal healthy control individuals age 45 years and older without any history of neurological disease.

4.4 Randomization and Blinding

These experiments were not randomized nor was blinding employed.

4.5 Study Assessments

The primary outcome is α4β2* nAChR receptor occupancy, defined as the difference between [^{18}F]Flubatine binding before and VCN discontinuation, i.e., 10 days after initiation of VCN administration (Day 10) and 5 days after VCN discontinuation (Day 15). Specifically, receptor occupancy is defined as [(Day 10 – Day 15) / Day 15] x 100%.

Adverse events were also collected throughout the 15-day study period.

5 Sample Size

The sample size (planned initially at four PD participants per dosing group) for the VCN- α4β2* nAChR occupancy study was based on logistical considerations. The selection of 10 control participants was also based on logistical considerations.

6 General Analysis Considerations

6.1 Timing of Analyses

The final analysis was completed after all all PD and control participants completed their follow-up.

6.2 Analysis Populations

All consented participants who received at least one dose of study medication will be used in the analyses of demographic and baseline characteristics and safety. Only participants who were able to complete sufficient treatment and PET images were included in the analyses of VCN- α4β2* nAChR occupancy.

6.3 Covariates and Subgroups

No covariates nor subgroups were incorporated into analyses.

6.4 Missing Data

No imputation methods will be used.

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 subject disposition, study conduct and safety data approximately every 6 months.

7 Summary of Study Data

Descriptive summary statistics will be derived for all data at baseline, separately by cohort (i.e., PD and control participant groups) and overall.

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 after initiation of study medication administration and the reasons for dropout will be summarized.

7.2 Protocol Deviations

Listings of protocol deviations will be provided by study cohort.

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) will be provided by study cohort.

8 Efficacy Analyses

Descriptive statistics for receptor occupancy will be provided by study cohort and by dose group.

9 Safety Analyses

Safety endpoints will be analyzed in all randomized participants who received at least one dose of study medication. Safety data, including adverse events (AEs) and the Columbia-Suicide Severity Rating Scale (C-SSRS), will be summarized descriptively by study cohort and by dose group.