**Clinical Study Protocol**
**Systemic Synuclein Sampling Study (S4)**

**PROTOCOL NO.:** S4-001

**INVESTIGATIONAL PHASE:** Observational

**Co-PRINCIPALINVESTIGATORS:**

**Clinical/Study Coordinating**
Lana Chahine, MD
Assistant Professor of Neurology
University of Pennsylvania
330 S 9th St
Philadelphia, PA
215-829-3038 (phone)
215-829-6606 (fax)

**Scientific**
Brit Mollenhauer, MD
Professor of Neurology
Paracelsus-Elena-Klinik, Kassel
University Medical Center Goettingen
Robert-Koch-Straße 40
37075 Goettingen, Germany
011-49-561-6009-200 (phone)
011-49-561-6009-126 (fax)

**SPONSOR/FUNDING:** The Michael J. Fox Foundation for Parkinson's Research
498 Seventh Avenue, 18th Floor
New York, NY 10018
212-509-0995 (phone)
212-509-2390 (fax)

**ORIGINAL VERSION DATE:** Version 4.0.; Dated October 18, 2016

# CONTENTS

# STUDY CORE FACILITIES AND FACILITY CONTACT INFORMATION

## Biorepository Core

Indiana University
410 W. 10th Street (HS4021)
Indianapolis, IN 46202
317-278-1291 (phone)

Biorepository Lead:
Tatiana Foroud, PhD
tforoud@iu.edu

## Clinical and Statistical Core

Clinical Trials Statistical & Data Management Center
University of Iowa
2400 University Capitol Centre
201 S. Clinton
Iowa City, Iowa 52242
319-335-8446 (phone)

Clinical Core Lead
Dixie J. Ecklund, RN, MSN, MBA
Associate Director
319-335-8446 (phone)
dixie-ecklund@uiowa.edu

Statistical Core Lead
Christopher S. Coffey, PhD
Professor, Department of Biostatistics
319-384-4197 (phone)
christopher-coffey@uiowa.edu

## PROTOCOL APPROVAL SIGNATURES

This Clinical Study/Trial Protocol is approved by:

Signature: Lana M. Chahine Date: 10/26/16
Lana Chahine, MD
Co-Principal Investigator

Signature: BRM Date: 11/03/16
Brit Mollenhauer, MD
Co-Principal Investigator

Signature: Tatiana Foroud Date: 11/07/16
Tatiana Foroud, PhD
Biorepository Core Lead

Signature: Dixie J Ecklund Date: 11/07/16
Dixie Ecklund, RN, MSN, MBA
Clinical Core Lead

Signature: Christopher S. Coffey Date: 11/07/16
Christopher S. Coffey, PhD
Statistical Core Lead

Signature: Vanessa Arnedo Date: 11/07/16
Vanessa Arnedo, MPH
The Michael J. Fox Foundation for Parkinson's Research (Sponsor)

## INVESTIGATOR SIGNATURE PAGE

| | |
|---|---|
| **Protocol Number:** | S4-001 |
| **Protocol Title:** | Systemic Synuclein Sampling Study |
| **Protocol Version:** | Version 4.0; Dated 18 October 2016 |
| **Sponsor:** | The Michael J. Fox Foundation for Parkinson's Research |

I have carefully read this protocol, including all appendices, and the investigator's drug brochure (if applicable), and agree that it contains all the necessary information for conducting the study safely.

I will conduct this study in strict accordance with this protocol and according to the current Good Clinical Practice (GCP) regulations and guidelines [21 CFR (Code of Federal Regulations) Parts 11, 50, 54 and 56 and ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) Topic E6 (R1)], and local regulatory requirements. Any changes in procedure will only be made if necessary to eliminate immediate hazards and/or to protect the safety, rights or welfare of subjects.

I will provide copies of the protocol and all other information relating to the pre-clinical and prior clinical experience, which were furnished to me, to all physicians and other study personnel responsible to me who participate in this study. I will discuss this information with them to assure that they are adequately informed regarding the study drug and conduct of the study.

I will ensure that the drugs supplied to me for this study will be used only for administration to subjects enrolled in this study protocol and for no other purpose.

I agree to keep records on all subject information (case report forms, informed consent statements, drug shipment, drug return forms, and all other information collected during the study) in accordance with the current GCP, local and national regulations.

| | |
|---|---|
| Principal Investigator's Signature | Date |

Principal Investigator Name (Print)

## LIST OF ABBREVIATIONS AND DEFINITIONS

| | |
|---|---|
| AE | adverse event |
| α-syn | alpha-synuclein |
| CBC | complete blood count |
| CFR | Code of Federal Regulations |
| CRF | Case Report Form |
| CSF | cerebrospinal fluid |
| CTSDMC | Clinical Trials Statistical and Data Management Center |
| DBS | deep brain stimulation |
| eCRF | electronic Case Report Form |
| EDC | electronic data capture |
| GCP | Good Clinical Practice |
| GI | Gastrointestinal |
| HC | Healthy Control |
| HIPAA | Health Insurance Portability and Accountability Act |
| HSPP | Human Subject Protection Program |
| ICH | International Conference on Harmonization |
| IEC | Independent Ethics Committee |
| IMM | Independent Medical Monitor |
| IRB | Institutional Review Board |
| INR | International Normalized Ratio |
| LP | lumbar puncture |
| MDS-UPDRS | Movement Disorder Society Unified Parkinson Disease Rating Scale |
| MJFF | The Michael J. Fox Foundation for Parkinson's Research |
| MoCA | Montreal Cognitive Assessment |
| OTC | over-the-counter |
| PD | Parkinson's disease |
| PPMI | Parkinson's Progression Markers Initiative |
| PT/PTT | prothrombin time/partial thromboplastin time |
| QA | quality assurance |
| QC | quality control |
| RBC | red blood cell |
| S4 | Systemic Synuclein Sampling Study |
| SAE | serious adverse event |
| SC | Steering Committee |
| SNc | substantia nigra pars compacta |

# SYNOPSIS

**TITLE**
Systemic Synuclein Sampling Study (S4)

**PROTOCOL Number S4-001**

**INVESTIGATOR/ STUDY CENTERS**
Approximately 6 clinical research sites in North America

**SPONSOR**
The Michael J. Fox Foundation for Parkinson's Research (MJFF)

**OBJECTIVES**

Primary Objective:

The goal of this study is to characterize the distribution of alpha-synuclein (α-syn) pathology through evaluation of quantitative and semi-quantitative outcomes for α-syn (total, phosphorylated, oligomeric and other α-syn species) in multiple tissues and body fluids in individual subjects with clinically typical Parkinson's disease (PD) and healthy controls (HC). The primary objectives are:

- To evaluate the α-syn markers as potential surrogate markers for patient selection/enrichment that would be useful in future clinical trials.
- To compare α-syn load and pattern in the biofluids and tissues in PD subjects versus HC subjects

Secondary Objectives:

- Evaluate the feasibility and safety of obtaining multiple tissues and biofluids in an individual with PD.
- Establish standardized protocols for biopsies of various tissues and methodology for α-syn staining and pathological analysis.
- Establish standardized protocols for various biofluid acquisition and assays for quantifying total α-syn and other α-syn species (once assays are available).
- Establish a bank of tissues and biofluids from a cohort of HC subjects and well-characterized subjects with typical PD for use for the future development and testing of biomarker assays.
- Compare the α-syn load among the tissues and fluids in the PD cohort subdivided by the clinical stage of disease (early PD not requiring dopamine replacement therapy, moderate PD on dopamine replacement therapy without motor fluctuations, advanced PD with motor fluctuations).
- Compare the α-syn load among the tissues and fluids with dopamine transporter (DAT) integrity based on striatal uptake measured by DAT imaging among the PD groups in addition to comparing PD versus HC.

**NUMBER OF SUBJECTS**
Up to 100 subjects will be enrolled to participate in this study, with a goal of enrolling 80 subjects (20 early PD not requiring dopamine replacement therapy, 20 moderate PD on dopamine replacement therapy without motor fluctuations, 20 advanced PD with motor fluctuations, 20 healthy controls) that complete at least 2 biofluid samplings [blood, saliva and cerebrospinal fluid (CSF)] and at least 2 tissue samplings (skin, colon, submandibular gland).

**ELIGIBILITY CRITERIA**

*Inclusion Criteria for PD Subjects*

- Male or female age 40 or older at the time of PD diagnosis.
- Clinical diagnosis of PD based on bradykinesia plus one of the following: rest tremor or rigidity.
- DAT deficit at screening based on visual interpretation of DaTSCAN™ imaging.
- PD subjects will need to fall into one of the following stages:
  - Early untreated PD not requiring dopamine replacement medication (anticholinergics, MAO-B inhibitors and amantadine permitted), Hoehn and Yahr 1-2, < 2 years from diagnosis.
  - Moderate PD responsive and currently treated with dopamine replacement therapy without evidence of motor fluctuations or dyskinesias.
  - Advanced PD with motor fluctuations or dyskinesias, > 5 years from diagnosis.
- Ability to provide written informed consent in accordance with Good Clinical Practice (GCP), International Conference on Harmonization (ICH), and local regulations.
- Willing and able to comply with scheduled visits, required study procedures and laboratory tests.

*Inclusion Criteria for HC Subjects*

- Male or female age 50 or older at the time of the screening visit
- Ability to provide written informed consent in accordance with Good Clinical Practice (GCP), International Conference on Harmonization (ICH), and local regulations.
- Willing and able to comply with scheduled visits, required study procedures and laboratory tests.

*Exclusion Criteria for all Subjects*

- Has a history of cancer (other than basal and squamous cell skin cancers), autoimmune disorder, liver disease, or other hematological disorder within the past 5 years.
- Current treatment with anticoagulants (e.g., Coumadin, heparin) that would preclude safe completion of the lumbar puncture (LP) and tissue biopsy procedures.
- Current treatment with an antiplatelet agent (Plavix or aspirin >325 mg/day).
- Has a diagnosis of diabetes mellitus requiring either an oral agent or insulin therapy.
- A bleeding diathesis, or clinically significant coagulopathy or thrombocytopenia.
- Has received botulinum toxin injections to the submandibular gland within the past year.
- Has a condition that precludes safe performance of routine LP, such as prohibitive lumbar spinal disease.

- Has a condition that precludes the safe performance of the flexible sigmoidoscopy procedure or may interfere with obtaining evaluable colonic tissue biopsies, including a prior colonoscopy with significant findings (e.g. polyp with a positive finding, ulcerative colitis, Crohn's disease, inflammatory disease).
- Has a condition that precludes the safe performance of the submandibular gland procedure or may interfere with obtaining evaluable submandibular tissue biopsies, including any previous or active significant disease affecting the submandibular gland (e.g. inflammatory disease, infection, tumor).
- Has a condition that precludes the safe performance of the skin punch biopsy procedure or may interfere with obtaining evaluable skin tissue biopsies, including any previous or active significant dermatological disease (e.g. previous biopsy with any of the following findings: inflammatory disease, scar tissue, psoriasis, keloid formation, skin cancer).
- Any other medical or psychiatric condition or laboratory abnormality, which in the opinion of the Site Investigator would preclude participation.
- Use of investigational drugs or devices within 30 days prior to the screening visit.

*Exclusion Criteria for PD Subjects*

- Has other significant neurological disorders (clinically significant stroke, brain tumor, hydrocephalus, epilepsy, other neurodegenerative disorders, encephalitis, repeated head trauma, polyneuropathy).
- Has significant autonomic dysfunction (symptomatic orthostasis, hypotension or urinary incontinence) suggestive of an atypical parkinsonism.
- Has atypical features of parkinsonism including but not limited to supranuclear gaze palsy, early recurrent falls, corticospinal track abnormalities, cerebellar abnormalities, significant cognitive dysfunction.

*Exclusion Criteria for HC Subjects*

- Has a family history of PD in any first-degree relative.
- Has a significant neurological disorder (a neurodegenerative condition, clinically significant stroke, brain tumor, hydrocephalus, epilepsy, other neurodegenerative disorders, encephalitis, repeated head trauma, polyneuropathy).
- Has a Montreal Cognitive Assessment (MoCA) score of less than 26.
- Has a diagnosis of REM sleep behavior disorder.
- Has a primary dystonia, restless legs syndrome, essential tremor, or other movement disorder.

**STUDY DESIGN**

This is a multi-center, cross-sectional, observational study to evaluate α-syn pathology in multiple tissues and biofluids in individual subjects with PD and HC at a single time point. Each subject's participation will be approximately 16 weeks. The total duration for sample collection for this study will be approximately 24 months.

**Eligibility Screening:** Screening activities will occur within 120 days prior to the tissue and biofluid collection visits. Subjects will sign the Informed Consent Form (ICF) and be assessed for study eligibility. The following will be collected at the screening visit to determine eligibility: demographics, family history of PD, medical history (including age of onset of PD), vital signs, concomitant medications, clinical laboratory evaluations (clinical chemistry, hematology, prothrombin and partial thromboplastin times (PT/PTT)), full physical examination, a standard 12-lead electrocardiography (ECG), MOCA, University of Pennsylvania Smell Identification Test (UPSIT), Scales for Outcomes in Parkinson's Disease - Autonomic (SCOPA-AUT), Movement Disorders Society Unified Parkinson Disease Rating Scale (MDS-UPDRS), Schwab and England Scale, Hoehn and Yahr stage, neurological examination, and PD Features Assessment. DaTSCAN™ imaging will be obtained to ensure DAT deficit for eligibility in PD subjects and to correlate with α-syn load in the biofluids and tissues. DaTSCAN™ imaging obtained as part of the Parkinson's Progression Markers Initiative (PPMI) study may be utilized for this study if completed within 6 months of the screening visit. Subjects that complete screening and are eligible based on the inclusion and exclusion criteria will be enrolled in the study.

**Biofluid Collection and Skin Biopsy Visit:**

- Whole blood, serum and plasma will be obtained and α-syn levels will be analyzed using the most currently available assays. Whole blood sample will also be used for extraction of RNA for analysis of α-syn transcripts. A blood sample will also be obtained for DNA extraction and genotyping. Additional nucleic acid and blood aliquots (whole blood, serum and plasma) will be banked for future investigation of PD biomarkers. A blood sample will also be obtained for a complete blood count (CBC) and assessment of reticulocytes for research purposes.
- CSF will be obtained and α-syn species levels will be analyzed using the most currently available assays. Additional CSF aliquots will be banked for future investigation of PD biomarkers.
- Saliva samples will be obtained using the passive drool collection method and analyzed for α-syn species levels using current, state-of-the-art assays. Additional saliva aliquots will be banked for future investigation of PD biomarkers.
- Skin punch biopsies will be performed under local anesthesia (lidocaine) on the cervical paravertebral region and distal thigh to evaluate for α-syn deposits in skin nerve fibers. Approximately 2 biopsies will be obtained at each location. Immunohistochemistry for α-syn and α-syn species will be performed by histopathology experts using currently available immunostains and techniques. Tissues not used in this analysis will be banked for future investigation of PD biomarkers.

**Colonic Biopsy Visit:**

- Colonic submucosa will be biopsied by un-prepped flexible sigmoidoscopy with optional conscious sedation using routine clinical procedure. Approximately 8 samples will be obtained from the sigmoid colon of each subject. The samples will be studied with immunohistochemistry for α-syn and α-syn species by histology experts using currently available immunostains and techniques. Tissues not used in this analysis will be banked for future investigation of PD biomarkers.

**Submandibular Gland Biopsy Visit:**

- Submandibular gland biopsies will be obtained using a 16-gauge needle under local anesthesia (lidocaine). Up to 4 attempts of needle core biopsies will be performed in each subject. Immunohistochemistry procedures for α-syn and α-syn species will be performed by histology experts using currently available immunostains and procedures. Tissues not used in this analysis will be banked for future investigation of PD biomarkers.

**SAFETY ASSESSMENTS**

Adverse events (AEs) will be collected and recorded throughout the reporting periods for each procedure, which is 7 days (± 2 days) following DaTSCAN™ imaging, lumbar puncture, skin biopsy, colonic biopsy and submandibular gland biopsy procedures.

**STATISTICAL METHODOLOGY**

**Sample Size Estimation:**

This is an exploratory trial. Data from the PPMI study (early PD cohort) can be used for the purposes of assessing the "effects" that this study may be powered to detect. In PPMI early PD subjects, the mean baseline total α-syn level in CSF was equal to 1845 ± 770 pg/ml. With a sample size of 20 subjects per group, and assuming a significance level of 0.05, we will have 80% power to detect a difference of 40% or more between HCs or one of the later PD cohorts versus the early PD cohort. Similarly, the study will have 90% power to detect a difference of 50% or more between HCs or one of the later PD cohorts versus the early PD cohort.

**Data Presentation/Descriptive Statistics:**

Descriptive statistics will be generated for demographic (age, gender, race, ethnicity) and clinical characteristics (duration of disease, MDS-UPDRS total and subscores, Hoehn and Yahr stage, Schwab and England, MOCA, SCOPA-AUT, UPSIT). For continuous variables, the mean, standard deviation, median, minimum, and maximum will be displayed for each subset of the PD cohort (20 early PD not requiring dopamine replacement therapy, 20 moderate PD on dopamine replacement therapy without motor fluctuations, 20 advanced PD with motor fluctuations), the PD subjects overall, and the HC cohort. For categorical variables, the percentage of subjects falling in each category will be displayed for each of these groups. Comparisons across the groups will be performed using t-tests and/or chi-square tests, as appropriate.

# 1. INTRODUCTION

## 1.1. Background

Parkinson's disease (PD) is pathologically characterized by the presence of Lewy bodies, composed of aggregated and phosphorylated alpha-synuclein (α-syn), in the subcortical regions of the brain (Spillantini et al, 1997). While the presence of Lewy bodies in the substantia nigra pars compacta (SNc) is a pathologically defining feature of PD, it is now well appreciated that α-syn pathology is not restricted to the central nervous system as it is also abundantly present in the peripheral nervous system. Extranigral α-syn pathology appears to precede the pathology in the SNc (Braak et al, 2003), which offers the potential for the development of a surrogate marker for early diagnosis through detection of α-syn in peripheral tissue or body fluid.

Alpha-synuclein has several isoforms as a result of alternative splicing of the *SNCA* gene. Alpha-synuclein 140 represents the whole transcript and retains all the sites that undergo post-translational processing and protein modification, making it the greatest contributor to aggregation of the protein (Beyer et al, 2013). The development of isoforms and further posttranslational modifications result in proteins with increased aggregation potential (McLean et al, 2002), oligomer formation and accumulation (Beyer et al, 2013). While the etiologies for α-syn aggregation are not well understood, environmental exposures and genetic mutations have been shown to induce misfolding of the protein (Breydo et al, 2012). The neurotoxic result of α-syn and its aggregates is malfunction of cellular processes and alteration in normal physiologic function (Bennett et al, 2005).

Alpha-synuclein has been visualized in neurons of the central and autonomic systems in biopsy tissue from the colon, salivary glands, and skin obtained from individuals with PD. Specifically, biopsy tissue from GI mucosa has demonstrated phosphorylated α-syn immunoreactive neurites in the submucosa of ascending colon of 80% of PD patients (n=5) (Lebouvier et al, 2008) and in the submucosal plexus of both the ascending and descending colon in 72% of PD patients (n=29) (Lebouvier et al, 2010). In a study focused on early, untreated PD subjects, 90% of patients demonstrated immunostaining for α-syn in the samples obtained from the sigmoid colon (n=10), compared to no detectable α-syn in controls (n=23) (Shannon et al, 2012). Based on these studies there appears to be reasonably high sensitivity for phosphorylated α-syn immunostaining in colon tissue, though the specificity is quite variable with positive staining in healthy subjects for some of these cohorts (Lebouvier et al. 2010; Visanji et al 2015).

Studies evaluating α-syn burden in salivary glands have evaluated labial salivary glands (minor salivary glands) and detected α-syn in approximately2/3 (67%) of PD cases (Cersosimo et al, 2011), however other studies have demonstrated 1/15 (6.7%) of α-syn in minor salivary glands (Adler et al, 2014). In a large post-mortem study, the submandibular gland was biopsied

demonstrating Lewy bodies in 89.5% of PD patients (n=17/19) and none in healthy controls (HC) (Beach et al, 2013), which was well tolerated resulting in only mild adverse events (AEs) making this a feasible procedure for tissue confirmation of PD. Only one study has performed an evaluation of α-syn in biopsies from HC subjects and 2/9 were positive for α-syn (Adler et al 2016) while no post-mortem cases were found to be positive (Beach et al, 2010; Del Tredici et al, 2010) and therefore this needs to be expanded to understand the specificity of α-syn immunostaining for PD.

Skin biopsy samples have been found to have a variable rate of α-syn detection from biopsies obtained in the extremities and buttocks (Mitchell et al, 2005), though a more recent study has shown more proximal biopsies from the cervical paravertebral region demonstrate phosphorylated α-syn in 100% of PD patients (n=21) compared to none of the HCs (n=30) or disease controls (n=20) (Donadio et al, 2014). There are several additional studies that have explored phosphorylated α-syn demonstrating variable sensitivity, often related to limited associations with dermal nerve fibers and modest specificity due to phosphorylated α-syn immunoreactive signal in both PD and HCs (Wang et al, 2013; Doppler et al, 2014; Dabby et al, 2006; Rossi et al, 2007). These recent studies provide justification for further study of skin biopsies from proximal sites as a potential peripheral maker for α-syn in both PD and HC subjects.

Studies have shown that α-syn is present in biofluids, including blood, saliva and cerebrospinal fluid (CSF) (Yanamandra et al, 2011; Devic et al, 2011; Barbour et al, 2008) and therefore assays measuring concentration differences for total, phosphorylated, and oligomeric synuclein levels in these fluids have been developed and showed largely decreased values for the total and increased values for phosphorylated α-syn and oligomeric α-syn in CSF in subjects with PD (Mollenhauer et al, 2005; 2011; Tokuda et al, 2006; Hong et al, 2010; Shi et al, 2011; El-Agnaf, 2006). Further data on the presence of other α-syn species in CSF is currently being generated by mass spectrometry and further assay development and optimization is ongoing. The lack of consistency in the CSF studies may be attributed to assay and antibody differences, different processing of samples and the contamination of the CSF with red blood cells (RBCs), which few of the studies accounted for. Alpha-synuclein levels in blood are highly abundant, especially in RBCs (Scherzer et al, 2007). Conflicting results have been reported for plasma α-syn levels with two studies reporting elevated α-syn in PD (El-Agnaf et al, 2006; Lee et al, 2006), one with decreased levels (Li et al, 2007) and one with no difference (Park et al 2011). The variability in these results may be related to the range in methodology used in these studies.

## 1.2. Study Rationale

The search for a safe, reliable and inexpensive biomarker of PD remains a major unmet need and is currently a high priority in PD research. The development of a peripheral α-syn biomarker would provide a valuable tool for confirming the diagnosis of PD and possibly identification of the disease in its earliest stages and provide a potential means of monitoring efficacy of potential disease modifying agents. Compared to other biomarkers for PD that are under investigation, many of which are related to dopaminergic neurons and are therefore influenced by the

compensatory responses related to dopamine deficiency, α-syn is expected to be more likely reflective of disease severity and progression as α-syn aggregation correlates with disease severity.

An important question that this study aims to address is what tissue or body fluid offers the most promising measure of α-syn load to determine if any of these tissue or biofluid sampling methods prove useful biomarkers for future use in therapeutic studies, particularly for those with disease modifying potential. The blood and saliva sampling procedures to be utilized as part of this study are of low risk and easily obtained. While collecting CSF is a more invasive procedure, this procedure is now being widely used for studies evaluating biomarkers for neurodegenerative diseases, including PD. The variability in the performance of the assays for measuring α-syn species in these biofluids is a major limitation. Evaluation of α-syn in peripheral tissues has the potential for more reliable outcomes. The tissue biopsies to be obtained as part of these studies involve routine biopsy procedures of peripheral tissues. Skin biopsies and sigmoid colon biopsies are procedures that are already performed routinely as part of preventive care in a large portion of the population. While submandibular gland biopsies are less frequently performed, the safety data in 40 PD subjects and 10 HCs undergoing biopsy have resulted in AEs mainly limited to swelling and bruising at the biopsy site (Adler et al 2015). In addition, given that the current literature suggests that α-syn pathology might begin in peripheral tissues in the pre-motor stage of disease and then move centrally to involve the central nervous system (CNS), understanding the peripheral α-syn burden may ultimately be key to the development of treatments to prevent progression into the CNS.

While all studies conducted thus far have utilized small sample sizes evaluating single biofluid or tissue types and using a variety of methodologies for sample collection, processing and analysis, the Systemic Synuclein Sampling Study (S4) design provides the opportunity to evaluate α-syn species in multiple tissues and biofluids within the same subject. S4 will also employ standardized collection, processing and analysis methodology across sites, making this the largest PD cohort evaluated for *in vivo* tissue sampling. Understanding the α-syn burden along the spectrum of disease severity (early untreated to advanced disease) will inform the next steps for utilizing these markers in the drug development process.

Disease severity, defined in S4 by both clinical scales and dopamine transporter (DAT) imaging, will be correlated with α-syn burden among multiple biofluids and tissues. While clinical assessments provide a measure of functionality, the PD motor scores are confounded by the symptomatic benefit of the dopamine replacing medications, even when implemented in a practically defined “off” state. DAT imaging offers an additional measure of disease severity, which is not confounded by the medications.

## 1.3. Ethical Rationale

There is no benefit expected for individual subjects participating in this study. The risk to subjects in this trial will be minimized by specialty training for practitioners completing the procedures, adherence to the inclusion/exclusion criteria and close clinical monitoring. There may be unknown risks to the procedures (colonic biopsy, submandibular gland biopsy, skin biopsy, lumbar puncture (LP) procedure, and phlebotomy), but given that these are standard procedures, most of which are used for health screening, and that there will be specialized training, the risk should be minimized. The potential benefit of this study, the development of markers of α-syn burden, provides the potential of serving as diagnostic tools in addition to objective measures of disease progression and thus filling a critical unmet need for PD research. Development of effective biomarkers would enhance the development of more effective therapeutics for the treatment of PD.

# 2. STUDY OBJECTIVES

## 2.1. Primary Objective

The goal of this study is to characterize the distribution of α-syn pathology through evaluation of quantitative and semi-quantitative outcomes for α-syn (total, phosphorylated, oligomeric and other α-syn species) in multiple tissues and body fluids in individual subjects with clinically typical PD and HC. The primary objectives for this study are as follows:

- To evaluate the α-syn markers as potential surrogate markers for patient selection/enrichment that would be useful in future clinical trials.
- To compare α-syn load in the biofluids and tissues in PD subjects with HC subjects

## 2.2. Secondary Objectives

The secondary objectives for this study are as follows:

- Evaluate the feasibility and safety of obtaining multiple tissues and biofluids in an individual with PD.
- Establish standardized protocols for biopsies of various tissues and methodology for α-syn staining and pathological analysis.
- Establish standardized protocols for various biofluid acquisition and assays for detecting total α-syn and α-syn species.
- Establish a bank of tissues and biofluids from a cohort of HC subjects and well-characterized subjects with typical PD for use for the future development and testing of biomarker assays.
- Compare the α-syn load among the tissues and fluids in the PD cohort subdivided by the clinical stage of disease (early PD not requiring dopamine replacement therapy,

moderate PD on dopamine replacement therapy without motor fluctuations, advanced PD with motor fluctuations).
- Compare the α-syn load among the tissues and fluids with DAT integrity based on striatal uptake measured by DAT imaging among the PD groups in addition to comparing in PD versus HC.

# 3. STUDY DESIGN

## 3.1. Overall Study Design

This is a multi-center, cross-sectional, observational study to evaluate α-syn pathology in multiple tissues and biofluids in individual subjects with PD and HC at a single time point. Each subject's participation will be approximately 16 weeks. The total duration for sample collection for this study will be approximately 24 months. Analysis of the samples will occur following collection of the samples.

The study is designed to occur in four visits: Screening Visit, Biofluid Collection and Skin Biopsy Visit, Colon Biopsy Visit and Submandibular Gland Biopsy Visit.

The Screening Visit will include obtaining informed consent prior to the initiation of any procedures followed by procedures to evaluate eligibility (clinical laboratory evaluations, medical history, physical examination, electrocardiography [ECG] and DaTSCAN™ Imaging) for participation, Montreal Cognitive Assessment (MoCA) and clinical characterization of PD (Movement Disorder Society Unified Parkinson's Disease Rating Scale [MDS-UPDRS], Hoehn and Yahr, Schwab and England, SCOPA-AUT). In addition, all subjects will complete a University of Pennsylvania Smell Identification Test (UPSIT). DaTSCAN™ imaging obtained as part of the Parkinson's Progression Markers Initiative (PPMI) study may be utilized for this study if completed within 6 months of the screening visit.

Upon confirmation of eligibility and within 120 days of the Screening Visit (based on the date of initial screening activities if occurs on more than one day), subjects will undergo the remaining visits: Biofluid Collection and Skin Biopsy Visit, Colon Biopsy Visit and Submandibular Gland Biopsy Visit. These visits may occur in an order most convenient for scheduling purposes.

For the Biofluid Collection and Skin Biopsy Visit, subjects will undergo collection of whole blood, serum, and plasma for the level of total α-syn, α-syn species and other potential biomarkers. Whole blood sample will also be used for extraction of DNA for genomic analyses and RNA for gene expression analyses. Whole blood sample will also be used for a complete blood count (CBC) and assessment of reticulocytes at a central laboratory. Saliva samples will be obtained using the passive drool collection method and analyzed for α-syn species levels using

current, state-of-the-art assays. Additional saliva aliquots will be banked for future investigation of PD biomarkers. An LP will be performed for CSF collection for assessment of the level of total α-syn, other α-syn species and additional potential biomarkers. Approximately 2 punch biopsies of the skin will be obtained under local anesthesia (lidocaine) at 2 locations (4 total biopsies), cervical paravertebral region (n=2) and distal thigh (n=2), to evaluate for α-syn burden in skin nerve fibers using the most current immunohistochemistry procedures. Skin samples not used for the initial analysis will be banked for future investigation of PD biomarkers.

For the Colonic Biopsy Visit, a flexible sigmoidoscopy will be performed (with or without conscious sedation) to enable approximately 8 biopsies of the sigmoid colon. The procedure will be performed by a gastroenterology collaborator identified by each clinical site in the available facility (office procedure room or endoscopy suite). The α-syn burden will be evaluated utilizing the most current immunohistochemistry techniques. Colon biopsy samples not used for the initial analysis will be banked for future investigation of PD biomarkers.

For the Submandibular Gland Biopsy Visit, up to 4 attempts of needle core biopsies will be obtained under local anesthesia (lidocaine). The α-syn burden will be evaluated utilizing the most current immunohistochemistry techniques. Submandibular gland samples not used for the initial analysis will be banked for future investigation of PD biomarkers.

AEs will be collected and recorded throughout the reporting periods for each procedure, which is 7 days (± 2 days) following DaTSCAN™ imaging, LP, skin biopsy, colonic biopsy and submandibular gland biopsy procedures.

## 3.2. Study Setting

The subjects will be recruited by approximately 6 PD clinical research centers in North America. All study procedures will be conducted at local specialty clinics (Otolaryngology, Gastroenterology, Nuclear Medicine, Neurology) identified by the clinical sites.

## 3.3. Study Population

### 3.3.1. Subject Numbers

Up to 100 subjects will be enrolled in this study. Subjects are considered enrolled in the study if they complete screening and are found to be eligible based on the inclusion and exclusion criteria. The enrollment goal for this study is 80 subjects (20 early PD not requiring dopamine replacement therapy, 20 moderate PD on dopamine replacement therapy without motor fluctuations, 20 advanced PD with motor fluctuations, 20 HC subjects) that complete at least 2 biofluid samplings (blood, saliva and CSF) and at least 2 tissue samplings (skin, colon, submandibular gland).

### 3.3.2. Inclusion Criteria for PD Subjects

- Male or female age 40 or older at the time of PD diagnosis.

- Clinical diagnosis of PD based on bradykinesia plus one of the following: rest tremor or rigidity.
- DAT deficit at screening based on visual interpretation of DaTSCAN™ imaging.
- PD subjects will need to fall into one of the following stages:
    - Early untreated PD not requiring dopamine replacement medication (anticholinergics, MAO-B inhibitors and amantadine permitted), Hoehn and Yahr 1-2, < 2 years from diagnosis.
    - Moderate PD responsive and currently treated with dopamine replacement therapy without evidence of motor fluctuations or dyskinesias.
    - Advanced PD with motor fluctuations or dyskinesias, > 5 years from diagnosis.
- Ability to provide written informed consent in accordance with Good Clinical Practice (GCP), International Conference on Harmonization (ICH), and local regulations.
- Willing and able to comply with scheduled visits, required study procedures and laboratory tests.

### 3.3.3. Inclusion Criteria for HC Subjects

- Male or female age 50 or older at the time of the screening visit
- Ability to provide written informed consent in accordance with GCP, ICH, and local regulations.
- Willing and able to comply with scheduled visits, required study procedures and laboratory tests.

### 3.3.4. Exclusion Criteria for all Subjects

- Has a history of cancer (other than basal and squamous cell skin cancers), autoimmune disorder, liver disease, or other hematological disorder within the past 5 years.
- Current treatment with anticoagulants (e.g., Coumadin, heparin) that would preclude safe completion of the lumbar puncture and tissue biopsy procedures.
- Current treatment with an antiplatelet agent (Plavix or aspirin ≥325 mg/day).
- Has a diagnosis of diabetes mellitus requiring either an oral agent or insulin therapy.
- A bleeding diathesis, or clinically significant coagulopathy or thrombocytopenia.
- Has received botulinum toxin injections to the submandibular gland within the past year.
- Has a condition that precludes safe performance of routine LP, such as prohibitive lumbar spinal disease.
- Has a condition that precludes the safe performance of the flexible sigmoidoscopy procedure or may interfere with obtaining evaluable colonic tissue biopsies, including a prior colonoscopy with any significant finding (e.g. polyp with a positive finding, ulcerative colitis, Crohn's disease, inflammatory disease).

- Has a condition that precludes the safe performance of the submandibular gland procedure or may interfere with obtaining evaluable submandibular tissue biopsies, including any previous or active significant disease affecting the submandibular gland (e.g. inflammatory disease, infection, tumor).
- Has a condition that precludes the safe performance of the skin punch biopsy procedure or may interfere with obtaining evaluable skin tissue biopsies, including any previous or active significant dermatological disease (e.g. previous biopsy with any of the following findings: inflammatory disease, scar tissue, psoriasis, keloid formation, skin cancer).
- Any other medical or psychiatric condition or laboratory abnormality, which in the opinion of the Site Investigator would preclude participation.
- Use of investigational drugs or devices within 30 days prior to the screening visit.

### 3.3.5. Exclusion Criteria for PD Subjects

- Has other significant neurological disorders (clinically significant stroke, brain tumor, hydrocephalus, epilepsy, other neurodegenerative disorders, encephalitis, repeated head trauma, polyneuropathy).
- Has significant autonomic dysfunction (symptomatic orthostasis, hypotension or urinary incontinence) suggestive of an atypical parkinsonism.
- Has atypical features of parkinsonism including but not limited to supranuclear gaze palsy, early recurrent falls, corticospinal track abnormalities, cerebellar abnormalities, significant cognitive dysfunction.

### 3.3.6. Exclusion Criteria for HC Subjects

- Has a family history of PD in any first-degree relative.
- Has a significant neurological disorder (a neurodegenerative condition, clinically significant stroke, brain tumor, hydrocephalus, epilepsy, other neurodegenerative disorders, encephalitis, repeated head trauma, polyneuropathy).
- Has a MoCA score of less than 26.
- Has a diagnosis of REM sleep behavior disorder.
- Has a primary dystonia, restless legs syndrome, essential tremor, or other movement disorder.

### 3.3.7. Enrollment Monitoring for Age, Gender and Disease Stage

Enrollments will be monitored centrally by the Steering Committee (SC) with the goal of achieving age and gender balance across the PD and HC subjects. In addition, the PD enrollment will be monitored for stage to ensure that the three stages (early untreated, moderate and advanced) are equally represented. Sites will be instructed if recruitment restrictions need to be implemented as the study progresses in order to maintain a balanced population and obtain the appropriate PD subjects.

### 3.3.8. Exclusionary Medications

Subjects taking anticoagulants and antiplatelet treatment (Plavix or aspirin >325 mg/day) will be excluded from participation. Given that study participation is elective, subjects should not discontinue or hold their anticoagulant or antiplatelet therapy in order to participate in the study.

Drugs that bind to the DAT with high affinity may interfere with the image obtained using ioflupane-123I (DaTSCAN™), which also binds to the dopamine transporter and are therefore required to be held for a period of at least 8 hours prior to the injection of DaTSCAN™. These drugs include amoxapine, amphetamine, bupropion, buspirone, cocaine, diethylpropion, ketamine, mazindol, methamphetamine, methylphenidate, modafinil, pemoline, phencyclidine (PCP), phentermine, phenylpropanolamine, pipradrol, selegiline, and sertraline.

## 3.4. Criteria for Termination of the Study

If the Sponsor, Principal Investigator, study monitor, or regulatory officials discover conditions arising during the study that indicate that the study should be halted or that the study site should be terminated, this action may be taken after appropriate consultation between the Sponsor and Principal Investigator. Conditions that may warrant termination of the study include, but are not limited to, the following:

- The discovery of an unexpected, serious, or unacceptable risk to the subjects enrolled in the study,
- A decision on the part of the Sponsor to suspend or discontinue testing, evaluation,
- Inability of the site to enroll subjects into the study at an acceptable rate,
- Inability of the site to complete timely data entry,
- Inability of the Site Investigator to comply with regulatory requirements,
- Insufficient adherence to protocol requirements

Study termination and follow-up will be performed in compliance with the conditions set forth in the following sections of the Code of Federal Regulations: 21 CFR 312.50 and 21 CFR 312.56.

## 3.5. Replacement of Subjects

Subjects that fail the screening procedures will be replaced. The data for this study is most valuable in subjects that have multiple biofluids and tissues for comparison. Up to 100 subjects will be enrolled in this study. The goal of the study is to enroll at least 80 subjects who are evaluable – defined as completing at least 2 biofluid samplings (blood, saliva, and CSF) and at least 2 tissue samplings (skin, colon, submandibular gland).

# 4. INVESTIGATIONAL PLAN

## 4.1. Subject Identification Numbers

A Subject ID Number will be assigned within the Electronic Data Capture (EDC) System by the Clinical Trials Statistical and Data Management Center (CTSDMC). This 6-digit number for PD and HC subjects will be used to identify the subject on all study forms and lab specimens.

## 4.2. Schedule of Activities (See Appendix 1)

## 4.3. Study Procedures by Visit

Subjects will undergo all procedures as outlined in the sections below. Assessments that require completion by the Site Investigator (unless otherwise approved and delegated) include: Neurological exam, MDS-UPDRS Part I, III and IV, Hoehn and Yahr Stage, and PD Stage Assignment.

Specific procedures for the clinical labs, biomic labs, imaging, LP, skin biopsy, colon biopsy and submandibular gland biopsy are provided in the S4 Laboratory Manual.

### 4.3.1. **Screening Visit**

All subjects will undergo a screening evaluation prior to the sampling visits. The procedures for the screening visit may take up to 8 hours and may occur over multiple days. The screening visit will include the following activities:

- An explanation of the purpose, procedures, potential risks and benefits of this study and informed consent will be obtained
- Review of the subject's medical and family history
- Review of concomitant medications
- Vital signs (blood pressure, heart rate, respiratory rate and temperature)
- General physical examination including height and weight
- General neurological examination
- MDS-UPDRS Parts I-IV
    - Part IV will not be done for healthy controls or early PD subjects
    - If not convenient to complete at Screening, MDS-UPDRS may be completed at any visit during the study
- Hoehn and Yahr (all subjects)
- Modified Schwab & England Activities of daily Living Scale (PD subjects only)
- PD Stage Assignment (PD subjects only)
- The Montreal Cognitive Assessment (MoCA) (all subjects)
- Scales for Outcomes in Parkinson's Disease-Autonomic (SCOPA-AUT) (all subjects)
- UPSIT (all subjects)
- Clinical laboratory assessments (Chemi-20 panel, CBC, PT/ PTT and serum pregnancy test for women of childbearing potential)

- Vital signs (blood pressure, heart rate, respiratory rate and temperature) prior to injection of DaTSCAN™ and at completion of the DaTSCAN™ imaging.
- DAT single photon emission computed tomography (SPECT) imaging (all subjects) (see Section 6.3.1), including urine pregnancy test for women of childbearing potential prior to injection
- ECG
- Review of AEs in follow up to SPECT imaging
- A review of the inclusion/exclusion criteria to confirm that the subject is eligible to continue to sampling visits

### 4.3.2. **Biofluid Collection and Skin Biopsy Visit**

The activities at this visit will be completed within 120 days of the Screening Visit (based on date of initial screening activities if occurs on more than one day). The Biofluid Collection and Skin Biopsy Visit will include the following activities and will take about 4 hours to complete. The procedures for this visit may occur over multiple days. The clinical coordinator will accompany the subject throughout the visit and will be responsible for collecting and processing biofluid and skin biopsy samples once the procedures are complete.

- Review of inclusion/exclusion criteria
- Review of concomitant medications
- Review of current medical conditions
- Blood draw for analysis of CBC and reticulocyte counts for research
- Blood draw for research samples (whole blood, serum, plasma) for analyses of α-syn species and other biomarker analyses
- Collect blood sample for DNA genomic analyses and RNA for gene expression analyses
- Collect saliva samples for analysis of α-syn species and other biomarker analyses
- Vital signs (blood pressure, heart rate, respiratory rate and temperature) prior to the initiation of LP and at completion of the LP procedure.
- LP for collection of CSF for analysis of α-syn species and other biomarker analyses
- Vital signs (blood pressure, heart rate, respiratory rate and temperature) prior to the initiation of skin biopsy and at completion of the skin biopsy procedure.
- Skin punch biopsies for skin tissue collection and staining for α-syn and other biomarker analyses
- Review of AEs related to LP
- Review of AEs related to skin biopsies

### 4.3.3. **Colon Tissue Collection Visit**

The activities at this visit will be completed within 120 days of the Screening Visit (based on the date of initial screening activities if occurs on more than one day). The Colon Tissue Collection Visit will include the following activities and will take about 1 hour to complete. This visit will

be completed on a single day. The biopsies will take place in a procedure room or facility utilized by the Gastroenterology collaborator at each site. The clinical coordinator will accompany the subject throughout the visit and will be responsible for collecting and processing tissue samples once the biopsies are complete.

- Review of the inclusion/exclusion criteria
- Review of concomitant medications
- Review of current medical conditions
- Vital signs (blood pressure, heart rate, respiratory rate and temperature) prior to initiation of the colon biopsy procedure and at completion of the colon biopsy procedure
- Flexible sigmoidoscopy and collection of sigmoid colon biopsies under optional conscious sedation for colon tissue collection and staining for α-syn and other biomarker analyses
- Vital signs (blood pressure, heart rate, respiratory rate and temperature) at completion of the procedure
- Review of AEs related to colon biopsy procedure

#### 4.3.4. **Submandibular Gland Tissue Collection Visit**

The activities at this visit will be completed within 120 days of the Screening Visit (based on the date of initial screening activities if occurs on more than one day). The Submandibular Gland Tissue Collection Visit will include the following activities and will take about 1 hour to complete. This visit will be completed on a single day. The biopsies will take place in a procedure room or facility utilized by the Otolaryngology collaborator at each site. The clinical coordinator will accompany the subject throughout the visit and will be responsible for collecting and processing tissue samples once the biopsies are complete.

- Review of the inclusion/exclusion criteria
- Review of concomitant medications
- Review of current medical conditions
- Vital signs (blood pressure, heart rate, respiratory rate and temperature) prior to initiation of the biopsy procedure and at completion of the submandibular gland biopsy procedure
- Submandibular gland needle core biopsies will be completed under local anesthesia for submandibular gland tissue collection and staining for α-syn and other biomarker analyses
- Review of AEs related to submandibular gland biopsy procedure

#### 4.3.5. **Follow-up Telephone Contacts**

Follow-up telephone visits will be conducted 7 days (±2 days) following a visit when DAT SPECT imaging, LP, skin biopsy, submandibular gland biopsy, and/or colonic biopsy have occurred. The purpose of the call is to assess for AEs.

#### 4.3.6. **Unscheduled Visits**

Unscheduled visits may be performed at any time during the study whenever necessary to assess for or to follow up on AEs or as deemed necessary by the Site Investigator or Coordinator. The following activities will be completed at an Unscheduled Visit:

- Vital signs
- *General physical examination
- *Collect blood for clinical laboratory assessments
- Review of current medical conditions
- Review of concomitant medications

*Conducted only if clinically indicated

# 5. STUDY PROCEDURES

## 5.1. Description of Study Assessments

#### 5.1.1. **Demographics**

Subject demographic and characteristic data to be collected on all subjects include: date of birth, gender, race and ethnicity. Relevant medical history and current medical conditions and medications will be recorded at screening.

#### 5.1.2. **MDS-UPDRS**

The MDS-UPDRS is a multimodal scale assessing both impairment and disability and is separated into 4 subscales (Parts I-IV). The MDS-UPDRS includes components assessed by the Site Investigator as well as sections completed by the subject.

- Part I: This assesses non-motor experiences of daily living and is comprised of two components:
  - Part IA contains 6 questions that are assessed by the Site Investigator and focuses on complex behaviors.
  - Part IB contains 7 questions that are part of the Patient Questionnaire completed by the subject.
- Part II: This assesses motor experiences of daily living. There are an additional 13 questions that are also part of the Patient Questionnaire completed by the subject.
- Part III: This assesses the motor signs of PD and is administered by the Site Investigator.
- Part IV: This assesses motor complications, dyskinesias and motor fluctuations using historical and objective information for PD subjects only. The Site Investigator will complete this assessment once a subject has started PD medication.

Subjects who have started PD medication (levodopa or dopamine agonist) will have an MDS-UPDRS and Hoehn and Yahr in a practically defined "OFF" state (after holding dopaminergic medications for a period of at least 6 hours). These assessments will then be repeated approximately one hour after receiving medication in clinic for a defined "ON" MDS-UPDRS motor exam (Part III only). These subjects will need to be reminded not to take PD medication on the day that the MDS-UPDRS visit will be performed.

### 5.1.3. Hoehn and Yahr Stage

The Hoehn and Yahr is a commonly used system for describing how the symptoms of PD progress. The scale allocates stages from 0 to 5 to indicate the relative level of disability. This scale is included within the MDS-UPDRS and will be completed for all subjects.

- Stage zero: No symptoms.
- Stage one: Symptoms on one side of the body only.
- Stage two: Symptoms on both sides of the body. No impairment of balance.
- Stage three: Balance impairment. Mild to moderate disease. Physically independent.
- Stage four: Severe disability, but still able to walk or stand unassisted.
- Stage five: Wheelchair-bound or bedridden unless assisted.

### 5.1.4. Modified Schwab and England (PD Subjects Only)

The Modified Schwab and England ADL scale reflects the speed, ease, and independence with which an individual performs daily activities, or personal chores, such as eating, toileting, and dressing. This scale uses a rating scale from 0% to 100%, with 100% representing complete independence in performing daily activities and 0% representing a vegetative, bedridden state.

### 5.1.5. Scales for Outcomes in Parkinson's Disease (SCOPA-AUT)

The SCOPA-AUT is a 26-item self-administered test developed to evaluate autonomic symptoms, such as GI and urinary problems, in subjects with PD.

### 5.1.6. University of Pennsylvania Smell Identification Test (UPSIT)

Odor identification performance will be assessed using the UPSIT (Doty et al, 1995), which will be self-administered at the Screening Visit. The UPSIT is a standardized, four-alternative, forced-choice test comprised of four booklets containing ten odorants apiece, one odorant per page. The stimuli are embedded in "scratch and sniff" microcapsules fixed and positioned on strips at the bottom of each page. A multiple-choice question with four-response alternative for each item is located above each odorant strip. Raw scores are calculated as the number of correct identifications. A percentile will be assigned based on comparison with age and gender matched controls.

### 5.1.7. The Montreal Cognitive Assessment (MoCA)

The MOCA was designed as a rapid screening instrument for mild cognitive dysfunction. It assesses different cognitive domains: attention and concentration, executive functions, memory,

language, visuoconstructional skills, conceptual thinking, calculations, and orientation. Time to administer the MoCA is approximately 10 minutes. The total possible score is 30 points; a score of 26 or above is considered normal. This scale will be performed at the clinical site as part of the baseline clinical characterization.

### 5.1.8. **Dopamine Transporter Imaging**

Subjects will have DAT imaging procedure to measure the amount of dopamine in the brain using SPECT. All subjects will undergo SPECT imaging as part of the screening.

The SPECT imaging procedure will be performed at the individual sites using DaTSCAN™.

Upon completion of the initial (Screening) SPECT scan, the imaging core will complete a Visual Interpretation Report. For PD subjects, the Visual Interpretation Report will be provided to the clinical site to inform eligibility. For the HC subjects, the Visual Interpretation Report will not be provided to the site since it does not impact eligibility.

- If the Visual Interpretation read for a PD subject indicates that the scan does not show evidence of DAT deficit, the subject will not be eligible for participation.
- In addition, a quantitative analysis will be completed to be compared with the α-syn biomarkers in the biofluid and tissue analyses.

Since this imaging information and the products used to complete the DAT SPECT scans are investigational as used in S4, it cannot provide definite information about a clinical diagnosis.

The procedure for DaTSCAN™ imaging includes:

Women of childbearing potential must have a urine pregnancy test prior to injection of DaTSCAN™. The result must be confirmed as negative prior to proceeding with the injection. Before the DaTSCAN™ injection, subjects will be pre-treated with stable iodine (10 drops of a saturated solution of potassium iodide) to reduce the uptake of DaTSCAN™ by the thyroid. Subjects will be injected with 3-5 mCi of dopamine transporter. Within a 4 hour (+/- 30 minute) window following the injection, subjects will undergo SPECT imaging on the camera. The data and quality assurance procedures to be employed in this study are described in the S4 Laboratory Manual.

Subjects will be monitored by study personnel for AEs on the day that a DaTSCAN™ is obtained. Subjects will also be contacted by phone 7 days (±2 days) following the injection/scan to assess adverse events. These events will be reported by the Site Investigator as required to the site's Institutional Review/Ethics Boards (IRB) and to his/her Radiation Safety Committee.

### 5.1.9. **Blood Sampling**

Whole blood (about 6 ml), serum (about 10 ml) and plasma (about 10 ml) will be collected to quantify total α-syn and α-syn species and also enable further exploratory biomarker analyses. An additional EDTA blood sample will be obtained (about 6 ml) for the extraction of DNA to conduct sequencing and genomic analyses. Blood will also be collected in PaxGene tubes (about 10 ml) for the extraction of RNA to conduct gene expression analyses. Additional blood (about 4 ml) will be collected to analyze CBC and reticulocyte counts. This additional blood for CBC and reticulocyte should be obtained on the same day or within 1-2 days of the research blood sampling. **It is strongly advised that the research blood samples are collected in a fasted state (i.e., minimum of 8 hours since last meal/food intake) to ensure the quality of samples for future analyses.** If fasting is not possible, then subjects should be strongly advised to eat a low lipid diet as provided. All research samples will be collected as described in the S4 Laboratory manual and sent to a central biorepository for analysis of blood cell counts for research purposes or to be stored for analysis of α-syn species. Samples will be made available to researchers with expertise in α-syn assays to conduct these analyses. Remaining samples may be used to evaluate other proteins, analytes or potential biomarkers. Subjects will not receive any individual results of analysis or testing conducted on the blood samples.

### 5.1.10. **Lumbar Puncture**

LP for CSF collection should be performed in the morning between 8-10am, preferably in a fasted state. If fasting is not feasible, then a low fat meal should be followed. The LP is performed by the Site Investigator or another qualified clinician appointed by the Site Investigator. An LP for the collection of 15-20 ml of CSF will be conducted for all subjects enrolled in the study. Prior to performing the LP, the Site Investigator will review the laboratory values to ensure that there is no evidence for a coagulopathy or other risk for bleeding. A fundoscopic examination or review of recent brain imaging will be performed to exclude conditions of increased intracranial pressure prior to performing the LP. The recommended procedure for performing the LP and collection of CSF is provided in the S4 Laboratory Manual. If difficulties obtaining spinal fluid occur and the subject is willing, an LP may be performed supported by fluoroscopy or ultrasound on the same or another day. The first 2 ml of CSF will be processed at the site's local lab facility to conduct standard analyses on white and red blood cell count, and total protein level. The remaining volume of CSF will be processed following the S4 Laboratory Manual and sent frozen to a central biorepository for storage. Samples will be made available to researchers with expertise in analysis of α-syn and/or other biomarker. Remaining samples may be used to evaluate other proteins, analytes or potential biomarkers. Subjects will be closely monitored during the procedure and following the procedure. Subjects will be contacted by phone $7 \pm 2$ days following an LP to assess for any AEs. Subjects will not receive any individual results of analysis or testing conducted on the CSF samples.

### 5.1.11. **Saliva Collection**

Saliva collection should occur at a study visit scheduled in the morning. Subjects should refrain from food intake, drinking liquids and using oral hygiene products for at least 1 hour prior to saliva collection. Prior to collection, the subject will rinse their mouth with water for 1 minute. After resting for a period of 5 minutes subjects will lean forward and hold their head down over a collection tube to initiate a passive drool. Saliva is collected over a period of 20 minutes or until 5 mL is obtained, whichever comes first. Saliva will be processed as described in the S4 Laboratory Manual. All research samples will be sent frozen to a central biorepository to be stored for analysis of α-syn species. Samples will be made available to researchers with expertise in α-syn assays to conduct these analyses. Remaining samples may be used to evaluate other proteins, analytes or potential biomarkers. Subjects will not receive any individual results of analysis or testing conducted on the saliva samples.

### 5.1.12. **Skin Biopsy**

Skin punch biopsies will be performed under local anesthesia (lidocaine) on the cervical paravertebral region and distal thigh to evaluate for α-syn deposits in skin nerve fibers. Approximately 2 biopsies will be obtained at each location (See S4 Procedure Manual for details regarding the skin biopsy procedure). Skin samples will be processed in formalin as described in the S4 Laboratory Manual and shipped to the central biorepository for storage and analysis of α-syn species. Immunohistochemistry for α-syn will be performed by histopathology experts using currently available immunostains and techniques. Remaining samples may be used to evaluate other proteins, analytes or potential biomarkers. Subjects will not receive any individual results of analysis or testing conducted on the skin samples. Subjects will be contacted by phone at 7 days (± 2 days) following the procedure to assess for any AEs.

### 5.1.13. **Colon Tissue Collection**

Colonic submucosa will be biopsied by unprepped flexible sigmoidoscopy with or without sedation (See S4 Laboratory Manual for details regarding the colon biopsy procedure). Approximately 8 samples will be obtained from the sigmoid colon of each subject. Samples from each subject will be fixed in formalin as described in the S4 Laboratory Manual and shipped to the central repository for storage and analysis of α-syn species. Immunohistochemistry for α-syn will be performed by histopathology experts using currently available immunostains and techniques. Remaining samples may be used to evaluate other proteins, analytes or potential biomarkers. Subjects will not receive any individual results of analysis or testing conducted on the colonic samples. Subjects will be contacted by phone at 7 days (± 2 days) following the procedure to assess for any AEs.

### 5.1.14. **Submandibular Gland Tissue Collection**

Submandibular gland biopsies will be obtained using a 16-gauge needle under local anesthesia (lidocaine) and optional ultrasound guidance. Up to 4 attempts of needle core biopsies will be performed in each subject. The biopsies will be performed unilaterally through a single puncture site for all biopsies. Tissue will be fixed in formalin as described in the S4 Laboratory Manual and shipped to the biorepository for storage and analysis of α-syn species. Immunohistochemistry for α-syn will be performed by histopathology experts using currently

available immunostains and techniques. Remaining samples may be used to evaluate other proteins, analytes or potential biomarkers. Subjects will not receive any individual results of analysis or testing conducted on the submandibular gland samples. Subjects will be contacted by phone at 7 days (± 2 days) following the procedure to assess for any AEs.

### 5.1.15. **Safety Assessments**

#### **5.1.15.1. Medical History**

Medical and family history, as well as a complete physical and neurological exam will be captured on all subjects at Screening.

#### **5.1.15.2. Adverse Events**

A clinical AE is any untoward medical occurrence in a subject undergoing procedures as a participant in a clinical trial, without regard to the possibility of a causal relationship. Study site personnel will record the occurrence and nature of each subject's pre-existing conditions. During the study, site personnel will record any change in the condition(s) and the occurrence and nature of any AEs that occur during the reporting periods, which occur 7 days (±2 days) after each of the following procedures: DaTSCAN™, LP, skin biopsy, colon biopsy and submandibular gland biopsy. Site Investigators will document their assessment of the potential relatedness of each AE to study procedures or imaging agent administration via an AE form.

#### **5.1.15.3. Clinical Laboratory Assessments**

A complete blood count (CBC), Chemistry-20 panel, and PT/PTT will be obtained at screening visit as noted on the schedule of activities. These laboratory assessments will be analyzed by the local laboratory at each of the sites. .For females of childbearing potential, a serum pregnancy test will be conducted at screening and a urine pregnancy test (urine dipstick) at the DaTSCAN™ imaging visit prior to injection with the radioligand.

In the case where a laboratory value is outside the reference range for the center at screening, a decision regarding whether the result is of clinical significance or not shall be made by the Site Investigator and shall be based, in part, upon the nature and degree of the observed abnormality. The assessment may be repeated once (for the purpose of inclusion) and in any case, prior to enrollment, to rule out laboratory error. In all cases, the Site Investigator will document the clinical considerations (i.e., result was/was not clinically significant and/or medically relevant) in allowing or disallowing the subject to continue in the study.

The laboratory testing will be made available to the practitioners that will be performing the procedures (LP, skin biopsy, colon biopsy and submandibular gland biopsy).

#### 5.1.15.4. Vital Sign Measurement

Analysis of safety parameters will include vital signs (blood pressure, heart rate, respiratory rate and temperature) at screening. In addition, vital signs (blood pressure, heart rate, respiratory rate and temperature) will be completed for safety purposes prior to initiation and at completion of the following procedures: DaTSCAN™, LP, skin biopsy, colon biopsy and submandibular gland biopsy.

#### 5.1.15.5. Electrocardiogram

The recording of a standard 12-lead ECG will be performed. A paper print-out of the ECG will be made and stored with the source documents at the sites. All ECGs must include the following information: paper speed, voltage calibration, lead identification for each lead, study number, subject number and date of birth, date and time of recording, and Site Investigator's signature following review of the ECG. The ECG will be interpreted locally. In all cases, the Site Investigator will document, the clinical considerations (i.e., result was/was not clinically significant and/or medically relevant) in allowing or disallowing the subject to participate in the procedures for this study. Paper ECGs may be subject to a study-specific central re-evaluation by an external cardiologist or a central ECG laboratory.

#### 5.1.15.6. Physical examination

A complete physical examination will include the examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, vascular and neurological. Height and weight will be measured and recorded at screening.

Information for the physical examination will be included in the source documentation at the study site and will be recorded. Significant findings that are present at the screening visit will be recorded in the Medical History. Significant findings made after completion of any study procedure, which meet the definition of an AE, will be recorded.

# 6. SAFETY REPORTING

## 6.1. Safety/Adverse Events

Site Investigators and coordinators will be instructed to assess for AEs at in-person study visits when DaTSCAN™ SPECT imaging, LP, skin biopsies, colon biopsies and submandibular gland biopsies are performed. AEs are also assessed by follow-up phone call at 7 days (±2 days) following all of these procedures.

Each subject must be carefully monitored for AEs. An assessment must be made of the seriousness, intensity, and relationship to the study procedure.

See table below for adverse event reporting periods:

| Procedure | Assessed through visit | 7 day (±2 days) telephone contact |
|---|---|---|
| Dopamine Transporter SPECT Imaging | X | X |
| Lumbar Puncture | X | X |
| Skin Biopsies | X | X |
| Colon Biopsies | X | X |
| Submandibular Gland Biopsies | X | X |

## 6.2. Adverse Event Definitions

It is the responsibility of the Site Investigator to document any AE that occurs during the reporting periods listed above in the study. An AE includes any noxious, pathological, or unintended change in anatomical, physiological, or metabolic functions as indicated by physical signs or symptoms occurring in any phase of the clinical study whether or not associated with the study procedures and whether or not considered related to the study procedures. This definition includes an exacerbation of pre-existing medical conditions or events, historical conditions not present prior to study treatment, which reappear following study treatment, intercurrent illnesses, hypersensitivity reactions, drug interaction, or the significant worsening of the disease under investigation that is not recorded elsewhere in the case report form (CRF). Anticipated day-to-day fluctuations of pre-existing conditions that do not represent a clinically significant exacerbation or worsening need not be considered AEs.

AEs during the reporting periods, whether observed by the Site Investigator, elicited from or volunteered by the subject, should be recorded on the AE Log and submitted to the CTSDMC via the Online AE Reporting System (AERS). This will include a brief description of the experience, the date of onset, the date of resolution, the severity, and seriousness and whether the event was related to a specific procedure or participation in this study.

AEs should not be solicited with leading questions that suggest specific signs or symptoms. Rather, AEs should be solicited by asking the subject a non-leading question such as: “Do you feel different in any way since undergoing the study procedure or since the last assessment?”

The Site Investigator will evaluate all AEs with regard to the maximum intensity and relationship to study procedures, as follows:

***Maximum intensity***

Maximum intensity should be assigned using 1 of the following 5 severity grades:

- Mild: aware of event but easily tolerated (Grade I)
- Moderate: discomfort, enough to cause interference with usual activity (Grade II)
- Severe: incapacitating: subject unable to work or perform usual activities (Grade III)
- Life-threatening (Grade IV)
- Death (Grade V)

***Relationship to study procedure***

The following will guide the judgment regarding the causality of an AE and determination if the AE is study procedure-related:

Not Related:

- Not Related -- Improbable temporal relationship and is plausibly related to concomitant drugs or underlying disease
- Unlikely: Occurred within a reasonable timeframe after study procedure, but there is a likely association of an intercurrent/underlying medical condition or concomitant drugs

Related:

- Possible – Occurred within a reasonable timeframe after study procedure, but could be related to concomitant drugs or underlying disease
- Probably – Occurred within a reasonable timeframe after study procedure, is unlikely to be attributable to concomitant drugs or underlying disease, and there is a plausible mechanism to implicate the study procedure
- Definite – Occurred within a reasonable timeframe after study procedure and cannot be explained by concomitant drugs or underlying disease

The degree of certainty with which an AE is attributed to the study procedures or alternative causes (e.g., natural history of the underlying diseases, concomitant therapy, etc.) will be determined by how well the event can be understood in terms of known effects of the procedures and/or reaction of similar nature being previously observed with the specific procedure in question.

Any AE ongoing at the final 7 days (±2 days) reporting telephone visit should be followed until resolution, but not more than 30 days following study participation.

## 6.3. Serious Adverse Events

An SAE is an AE that is fatal or life-threatening, or results in hospitalization, prolongation of hospitalization, persistent or significant disability/incapacity, or a congenital anomaly/birth defect. A life-threatening AE is an AE that, in the view of the Site Investigator, places the subject at immediate risk of death from the reaction, as it occurred. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Inpatient admission in the absence of a precipitating, procedure-related clinical adverse event is not subject to immediate reporting. For example:

- Admission for treatment of a pre-existing condition not associated with the development of a new AE
- Social admission (e.g., subject has no place to sleep)
- Protocol-specific admission during a clinical study (e.g., for a procedure required by another study protocol)
- Optional admission not associated with a precipitating clinical AE (e.g., for elective cosmetic surgery)

Inpatient admission does not include the following:

- Emergency Room/Accident and Emergency/Casualty Department visits
- Outpatient/same-day/ambulatory procedures
- Observation/short-stay units
- Rehabilitation facilities
- Hospice facilities
- Respite care (e.g., caregiver relief)
- Skilled nursing facilities
- Nursing homes
- Custodial care facilities

## 6.4. Recording of Adverse Events

AEs, whether observed by the Site Investigator, elicited from or volunteered by the subject, should be recorded on the AE Log and reported to the CTSDMC via AERS. AEs are to be submitted to the CTSDMC within 5 working days of the site becoming aware of the event. This will include a brief description of the experience, the date of onset, the date of resolution, the severity, and seriousness and whether the event was related to a specific procedure (procedure-related) in the research study.

Any AE ongoing at the final 7 days (±2 days) reporting telephone visit should be followed until resolution or stabilization, but not more than 30 days from completion of the last study procedure.

The Independent Medical Monitor (IMM) for this study will review all AEs in aggregate on a quarterly basis. The CTSDMC will provide quarterly reports to the IMM and request an evaluation. Any trends or events of concern will be provided to the Site Investigator and to the Sponsor.

## 6.5. Responsibilities for reporting Serious Adverse Events

- The Site Investigator will submit to the CTSDMC an AE Form through the AERS within 24 hours of his/her becoming aware of the occurrence of a SAE. The CTSDMC Project Manager will review the SAE and forward to the IMM for review.

- The following information should be supplied if available at the time of submission of the SAE study number, site number, subject number, subject age and gender, date of onset of event, event description, whether event required treatment, death and autopsy report, an identification of which criteria for a serious experience have been met, the Site Investigator's current opinion of the relationship between the event and study participation.

- The IMM will review the SAE within 2 working days of submission. The IMM will review whether the event meets the criteria for seriousness, relatedness, and expectedness. If needed, the IMM may consult with experts in the specific procedures for additional information regarding the procedures.

- The Site Investigator will comply with his/her local IRB and Radiation Safety Committee regulations regarding the reporting of AEs.

# 7. REPORTABLE EVENTS

The following incidents will be considered reportable events and will be reported to the CTSDMC within 24 hours of the event, or the Site Investigator's knowledge of the event.

- Change of clinical diagnosis
- Participation in an interventional/therapeutic clinical trial
- Premature withdrawal (e.g. withdrawal of consent)
- SAE
- Unanticipated problems that are not AEs
- Pregnancy (reported by female subject or female partner of a male subject)
- Death

# 8. REFERRALS FOR MEDICAL CARE

If a research assessment or laboratory value result reveals a clinically significant abnormality (e.g., depression, polyps requiring further assessment/biopsy, renal impairment on metabolic profile) the subject will be informed of this result and instructed to follow up with his or her primary care physician. Should there be a safety concern warranting a referral for medical or psychiatric follow-up, the Site Investigator should provide the subject with the appropriate referral as necessary.

# 9. POTENTIAL RISKS

## 9.1. Blood Sampling

Risks associated with venous blood draw include pain and bruising at the site where the blood is taken. Sometimes people can feel lightheaded or even faint after having blood drawn.

## 9.2. Lumbar Puncture

The most common risks of a LP are pain at the site and a temporary headache usually due to a small amount of CSF leakage around the needle insertion site. Lying down for a few hours after the test can make a headache less likely to occur. There is a slight risk of infection because the needle breaks the skin's surface, providing a possible portal of entry for bacteria. A temporary numbness to the legs, transient eye muscle palsy or lower back pain may be experienced. There is a small risk of bleeding in the spinal canal. Subjects will have blood drawn at Screening to test for coagulopathies.

## 9.3. DaTSCAN™ SPECT Imaging

Specific potential risks for DAT SPECT imaging are as follows:

1. Radiation exposure from DaTSCAN™
2. Potential pharmacological effects of DaTSCAN™,
3. Placement of an intravenous catheter. Risks associated with the intravenous injection include pain and bruising at the site where the Intravenous catheter is placed. Sometimes people can feel lightheaded or even faint after having an intravenous catheter placed.

Risks of DaTSCAN™: DaTSCAN™ is administered at radiotracer doses and is not expected to have any pharmacological or toxicological effects. The radiation of exposure from a single dose of DaTSCAN™ (85 MBq or 5 mCi) is approximately 0.4 rems. DaTSCAN™ binds to the dopamine and serotonin transporter. At pharmacologic doses DaTSCAN™ might be expected to have stimulant-like effects and affect cardiovascular responses. However, in the proposed study the estimated mass dose of DaTSCAN™ is very low - <30/pmol kg. More than 180,000 doses of the drug have been administered to human subjects.

Iodine: Prior to the DaTSCAN™ injection subjects will be pretreated with SSKI (saturated solution of potassium iodide) or another thyroid protective agent standardly used in each of the

nuclear medicine imaging centers. Potential side effects from SSKI include nausea, vomiting, stomachache, diarrhea, metallic taste in the mouth, fever, headache, or acne may occur. If any of these effects persist or worsen, tell your study doctor promptly.

In addition to the known risks listed above, the imaging procedure in this study may cause unknown risks to the participant, or a developing embryo or fetus or possible risks to the future offspring of male participants. Female subjects or a female partner of a male subject who report a pregnancy within 30 days of DaTSCAN™ injection will be asked to have a urine pregnancy test within 7 days of reporting the pregnancy.

## 9.4. Skin Biopsy

Risks associated with performing punch biopsies of the skin include pain and bruising at the site where the biopsy is taken. In addition, there may be blood loss at the site of the biopsy, which may require suturing. There is also a possibility of infection of the biopsy site if proper care is not provided. Sometimes people can feel lightheaded or even faint after having a skin biopsy performed.

## 9.5. Submandibular Gland Biopsy

Risks associated with performing submandibular needle biopsies include pain and bruising at the needle insertion site, bleeding or inflammation within submandibular gland, hematoma, or dysphagia. In addition, there may be blood loss at the site of the needle insertion that may require pressure dressing or in rare cases requiring cauterization. There is also the possibility of infection at the biopsy site or local numbness if a nerve is inadvertently exposed to the needle. Sometimes people can feel lightheaded or even faint after having a submandibular gland biopsy performed.

## 9.6. Flexible Sigmoidoscopy and Colon Biopsy

Risks associated with performing a flexible sigmoidoscopy and colon biopsies include pain at the site where the biopsy is taken, bleeding or inflammation within colon. In addition, there may be blood loss as a result of the biopsy procedure that may require further evaluation and in rare cases cauterization. There is also the possibility of infection at the biopsy site or perforation of the colon if the biopsy forceps extend beyond the colonic submucosa, which could require surgical repair. Sometimes people can feel lightheaded or even faint after having a flexible sigmoidoscopy and colonic biopsy performed.

# 10. STATISTICAL METHODOLOGY

## 10.1. Analysis Populations

10.1.1. **Safety Analysis Population:**

All safety analyses will include all subjects who were enrolled and completed at least one sampling procedure (LP, blood draw, saliva collection, skin

biopsy, colonic biopsy, or submandibular gland biopsy) as part of this study. Subjects in this analysis set will only be used for feasibility and safety analyses.

10.1.2. **Synuclein Burden Population:**

For analyses examining α-syn burden across groups, all subjects with relevant data for all comparisons will be used in the α-syn burden analyses.

## 10.2. Descriptive Statistics

Descriptive statistics will be generated for important demographic (age, gender, race, ethnicity) and clinical characteristics (duration of disease, MDS-UPDRS total and motor score, Hoehn and Yahr, Schwab and England, MOCA, SCOPA-AUT). For continuous variables, the mean, standard deviation, median, minimum, and maximum will be displayed for each subset of the PD cohort (20 early PD not requiring dopamine replacement therapy, 20 moderate PD on dopamine replacement therapy without motor fluctuations, 20 advanced PD with motor fluctuations), the PD subjects overall, and the HC cohort. For categorical variables, the percentage of subjects falling in each category will be displayed for each of these groups. Comparisons across the groups will be performed using t-tests and/or chi-square tests, as appropriate.

Demographic variables of screen failure subjects, and reasons for screen failures, will be summarized for all subjects who are screened but not randomized into the study.

## 10.3. Study Outcome Measures

10.3.1. **Biofluid α-syn levels:**

Whole blood, serum, plasma, saliva, and CSF α-syn will be analyzed using the most optimal, currently available, quantitative and semi-quantitative assays. The outcome will be expressed as a concentration of total α-syn and other α-syn species or a ratio of specific species to total levels.

10.3.2. **α-syn deposits in skin:**

α-syn burden in skin biopsies will be expressed as 1) simply positive or negative, i.e. whether any two slides are positive out of all examined 2) by total percentage of slides examined that are positive 3) by site of highest density of positive α-syn fibers.

10.3.3. **α-syn deposits in submandibular gland:**

α-syn burden in the submandibular tissue will be expressed as 1) simply positive or negative, i.e. whether any two slides are positive out of all examined 2) by total percentage of slides examined that are positive 3) by site of highest density of positive α-syn fibers.

#### 10.3.4. **α-syn deposits in colon:**

α-syn burden in the colon tissue will be expressed as 1) simply positive or negative, i.e. whether any two slides are positive out of all examined 2) by total percentage of slides examined that are positive 3) by site of highest density of positive α-syn fibers.

## 10.4. Analysis Plan

### 10.4.1. **Primary Objective**

The goal of this study is to characterize the distribution of α-syn pathology through evaluation of quantitative and semi-quantitative outcomes for α-syn (total, phosphorylated, oligomeric, and other α-syn species) in multiple tissues and body fluids in individual subjects with clinically typical PD and HC subjects. The primary objectives of the study are to:

- Evaluate the markers of α-syn as potential surrogate markers for patient selection/enrichment that would be useful in future clinical trials
- Compare α-syn load in the biofluids and tissues in PD subjects with HC subjects

To address the first objective, the distribution of each α-syn measurement will be closely examined. The variability of each α-syn measurement will also be closely examined in an attempt to make recommendations for the optimal measurement to consider in future clinical trials (i.e., the measurement with the lowest variability and, hence, smallest required sample size, to detect clinically important effects of interest).

Two sets of comparisons will be performed to assess the second objective. First, all PD subjects will be pooled and compared to the set of HC subjects with respect to each individual α-syn measurement. Then, subjects in each of the three separate PD groups will be compared to the healthy controls.

### 10.4.2. **Secondary Objective:**

**Feasibility & Safety:** One important secondary objective is to evaluate the feasibility and safety of obtaining multiple tissues and biofluids in an individual with PD. Feasibility will be assessed by tabulating the percentage of subjects in each group who provide complete samples. The assessment of safety will be based primarily on the frequency of procedure related AEs. All AEs will be coded using the Medical Dictionary for Regulatory Authorities (MedDRA). Procedure related AEs will be summarized by system organ class and preferred term. We will examine safety across groups in two different ways. First, the percentage of subjects who experience any procedure related AE will be compared across groups using a chi-square test. Additionally, the rates of procedure related AEs will be compared using a Generalized Linear Model with a Poisson link.

**Compare α-syn Load Across PD Groups:** Another important secondary objective is to compare the α-syn load among the tissues and fluids in the PD cohort subdivided by the clinical stage of disease (early PD not requiring dopamine replacement therapy, moderate PD on

dopamine replacement therapy without motor fluctuations, advanced PD with motor fluctuations). To address this objective, trend tests (continuous or categorical, as appropriate) will be performed to assess α-syn levels across the three cohorts of PD subjects.

**Compare α-syn Load with DAT Imaging:** Another important secondary objective is to compare the α-syn load among the tissues and fluids with disease severity based on striatal degeneration measured by DAT imaging across the PD groups in addition to comparing PD and HC groups. To accomplish this objective, each measure of α-syn load will be correlated with DAT imaging data using an appropriate Generalized Linear Model.

## 10.5. Sample Size Justification

As this is an exploratory trial, it is difficult to obtain solid estimates for key parameters of interest. However, the PPMI study provides data in an early PD cohort that can be used for the purposes of assessing the "effects" that this study may be powered to detect. In the PPMI cohort of early PD subjects, the mean baseline CSF levels of total α-syn was equal to 1845 ± 770 pg/ml. With a sample size of 20 subjects per group, and assuming a significance level of 0.05, we will have 80% power to detect a difference of 40% or more between HCs or one of the later PD cohorts versus the early PD cohort. Similarly, the study will have 90% power to detect a difference of 50% or more between HCs or one of the later PD cohorts versus the early PD cohort.

# 11. REGULATORY/ETHICS

## 11.1. Compliance Statement

This study will be conducted in accordance with the GCP and ICH guidelines and any applicable national and local regulations.

Laboratory tests/evaluations described in this protocol will be conducted in accordance with quality laboratory standards as described in the S4 Laboratory Manual unless otherwise stated.

## 11.2. Informed Consent

In accordance with relevant regulations, an informed consent agreement explaining the procedures and requirements of the study, together with any potential hazards/risks must be read by and/or explained to each subject. Each subject will sign such an informed consent form. The subject must be assured of the freedom to withdraw from participation in the study at any time.

It is the Site Investigator's responsibility to make sure that the subject understands what she/he is agreeing to and that written informed consent is obtained before the subject is involved in any protocol-defined procedures including screening procedures. It is also the Site Investigator's responsibility to retain the original signed consent form and provide each subject with a copy of the signed consent form.

The CTSDMC must be given an opportunity to review the consent forms prior to site IRB submission and before it is used in the study.

## 11.3. Institutional Review Board/Independent Ethics Committee

The CTSDMC will supply all necessary information to the Site Investigator for submission of the protocol and consent forms to the IRB/IEC for review and approval. The Site Investigator agrees to provide the IRB/IEC all appropriate material. The trial study will not begin until the Site Investigator has obtained appropriate IRB/IEC approval. A copy of the approval letter and approved consent form must be submitted to the CTSDMC.

The Site Investigator will request from the IRB/IEC a composition of the IRB members reviewing the protocol and informed consents. Appropriate reports on the progress of this study by the Site Investigator will be made to the IRB/IEC and the CTSDMC in accordance with institutional and government regulations. The CTSDMC will notify the site when the IRB/IEC may be notified of study completion. It is the Site Investigator's responsibility to notify the IRB when the study ends. This includes study discontinuation, whether it is permanent or temporary. A copy of the site IRB/IEC's acknowledgement of study completion must be submitted to the CTSDMC.

## 11.4. Protocol Amendments

Changes to the protocol should only be made via an approved protocol amendment. Protocol amendments must be approved by the Sponsor, the study's SC and each respective site's IRB/IEC prior to implementation, except when necessary to eliminate hazards and/or to protect the safety, rights or welfare of subjects.

## 11.5. Subject Confidentiality

The Site Investigator must assure that the confidentiality of subjects, including their personal identity and personal medical information, will be maintained at all times. In addition, the sites have confidentiality obligations to study subjects under the Health Insurance Portability and Accountability Act (HIPAA). Subjects will be identified by code numbers on case report forms and other study materials submitted to the CTSDMC, the central laboratory, and central biorepository. After a subject signs an informed consent, it is required that the Site Investigator permit the study monitor and/or other personnel from the CTSDMC or regulatory agency personnel to review the signed informed consent(s) and that portion of the subject's medical record that is directly related to the study. This shall include all study relevant documentation including subject medical history to verify eligibility, laboratory test result reports, admission/discharge summaries for hospital admissions occurring while the subject is in the study, and autopsy reports for deaths occurring during the study (when available).

# 12. DOCUMENTATION

## 12.1. Study File and Site Documents

The Site Investigator should have the following study documents accessible to the Monitor during the study. A comprehensive list of required essential documents for this study will be provided to the Site Investigator by the CTSDMC.

- *Curriculum vitae* for Site investigator and coordinator
- The signed IRB/IEC form/letter stating IRB/IEC approval of protocol, consent forms, and advertisement notices, documentation of the IRB/IEC composition, and all IRB/IEC correspondence including notification/approval of protocol amendments, notification of serious adverse events to the IRB/IEC, and IRB/IEC notification of study termination
- IRB/IEC approved consent forms (sample) and advertisements as applicable
- Signed protocol (and amendments, where applicable)
- Signed subject consent forms
- Copies of the completed CRF worksheets
- Delegation Log with names, signatures, initials and functional role of all persons completing protocol assessments, providing back-up to the Site Investigator and Coordinator, if applicable, as well as staff entering data to the electronic data capture system.
- Laboratory accreditation and relevant laboratory reference ranges
- Copies of laboratory reports/printouts
- Any source data/records not kept with the subject's hospital/medical records
- Signed and dated receipt of supplies
- Record of all monitoring visits
- Copies of correspondence to and from CTSDMC
- Investigator's Brochure (where applicable)
- Certificate for Human Subject Protection Program (HSPP) or equivalent GCP program for each individual named on the Delegation log who has direct subject contact
- Copy of professional licensure/registration, as applicable, for each individual who has direct subject contact ensuring licensure is in the state/region in which the study will be conducted
- A Note to File indicating the assessments that will be considered source documents
- Any other documentation as required by the CTSDMC (e.g., Conflict-of-Interest/Financial Disclosure)

The Site Investigator must also retain all printouts/reports of tests/procedures, as specified in the protocol, for each subject. This documentation, together with the subject's hospital/medical records, is the subject's source information for the study.

## 12.2. Maintenance and Retention of Records

It is the responsibility of the Site Investigator to maintain a comprehensive and centralized filing system of all relevant documentation. Site Investigators must retain all study records required by the CTSDMC and regulatory authorities in a secure and safe facility with limited access. The Site Investigator will be instructed to consult with the CTSDMC before disposal of any study records and to notify the CTSDMC of any change in the location, disposition, or custody of the study files.

An electronic CRF (eCRF) utilizing an EDC application will be used for this study. In the event of an audit or regulatory authority inspection, the eCRFs can be printed out.

## 12.3. Case Report Forms

Sites will enter subject information and data into the eCRF in the EDC application. The eCRFs are used to record study data and are an integral part of the study and subsequent reports. Therefore, the eCRFs must be completed according to the subject's source data on a per-visit basis for each subject screened or enrolled. Authorized study personnel will each be granted access to the EDC system via provision of a unique password-protected user-ID that will limit access to enter and view data specifically for subjects enrolled at their site. Timely data entry is considered to be data entered into the EDC system within 14 days of a subject's visit.

Sites will have access to download worksheets that correspond to the eCRF. The worksheets will serve as source documents (if applicable) as described in the Operations Manual and are to be used to enter data into the eCRFs. Sites will enter all data into the subject's medical chart and/or onto source documentation worksheets prior to entering data into the eCRFs.

It is the Site Investigator's responsibility to ensure that entries are proper and complete. During entry of data, error checks will be performed by the EDC system that will immediately flag problematic data (i.e., missing, out of range, inconsistent) allowing for sites to correct the data at that time. Error checks will be implemented in the EDC system based upon specifications defined in the data management plan.

## 12.4. Primary Source Documents

The Site Investigator must maintain primary source documents supporting data collected for each subject. This includes documentation of:

- General information supporting the subject's consent to participate in the study
- Demographic information
- Evidence supporting the diagnosis/condition for which the subject is being studied
- General history and physical findings
- Hospitalization or Emergency Room records (if applicable)
- Each study visit by date, including any relevant findings/notes by the Site Investigator(s), occurrence (or lack) of adverse events, and changes in medication usage including the date the medication commenced and completed
- Any additional visits during the study
- Any relevant telephone conversations with the subject regarding the study or possible adverse experiences

- Original, signed informed consent forms for study participation

During monitoring visits the study monitor will need to validate data in the eCRFs against these source data.

## 13. STUDY DATA MANAGEMENT

An Internet accessible EDC system for data management will be utilized for this study. This system is protected by 128-bit server certificates and utilizes authenticated, password-protected accounts for each site. The EDC system is designed to ensure timeliness and accuracy of data as well as the prompt reporting of data from the study on an ongoing basis to the Site Investigators. The system is compliant with relevant FDA regulatory requirements per 21 CFR Part 11.

Data review, coding and query processing will be done through interaction with the CTSDMC, site personnel and the Study Monitor. Queries will be generated in real-time as data are entered. Once the data are submitted to the EDC system, they are immediately stored in the central study database located at the CTSDMC and are accessible for review by data management staff. Any changes to the data will be fully captured in an electronic audit trail. As data recorded by sites in eCRFs are received, narrative text of adverse experiences and concomitant medications will be periodically coded using established coding mechanisms.

The cycle of electronic data entry, review, query identification/resolution, and correction occurs over the course of the study period until all subjects have completed the study.

At the end of the study, the CTSDMC will work in conjunction with the Sponsor and the Principal Investigator to adequately resolve all queries. Once all queries have been adequately resolved, the database will be deemed “clean” and officially “locked”. All permissions to make changes (append, delete, modify or update) to the database are removed at this time.

## 14. STUDY MONITORING

In accordance with ICH Guidelines for GCP 5.18 the study will be monitored to verify that:

(a) The rights and wellbeing of human subjects are protected.
(b) The reported trial data are accurate, complete, and verifiable from source documents.
(c) The conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirement(s).

The IMM and the SC have the responsibility to monitor all procedures for safety and for GCP and regulatory compliance. The committee members have the expertise to monitor all aspects of this study.

The study will have ongoing monitoring to ensure that the trial is conducted properly. The monitoring activities will include:

- Verifying that the Site Investigators and coordinators have adequate qualifications, that resources remain adequate throughout the trial period, and that facilities, equipment, and staff are adequate to safely and properly conduct the trial.
- Verifying that the Site Investigators follow the approved protocol and all approved amendment(s), if any.
- Verifying that written consent was obtained for each subject participating in the trial.
- Verifying that the Site Investigators are enrolling only eligible subjects.
- Verifying that source documents and other trial records are accurate, complete, kept up-to-date and maintained.
- Verifying that the Site Investigator provides all the required reports, notifications, applications, and submissions, and that these documents are accurate, complete, timely, legible, dated, and identify the trial.
- Monitoring AEs, concomitant medications and intercurrent illnesses.
- Determining whether all AEs are appropriately reported within the time periods required by GCP, the protocol, the IRB, and other applicable regulatory requirement(s).
- Communicating deviations from the protocol, Standard Operating Procedures, GCP, and the applicable regulatory requirements to the Site Investigator and taking appropriate action designed to prevent recurrence of the detected deviations.
- Federal regulations 21 CFR §56.109(f) and 45 CFR §46.109(e) state that an IRB shall conduct continuing review of research covered by these regulations at intervals appropriate to the degree of risk, but not less than once per year, and shall have authority to observe or have a third party observe the consent process and the research. Continuing review by the IRB routinely includes interim progress reports, as directed by the Board, review of proposed changes to research, adverse event reports, review of any protocol deviations, visits to the research site, and annual review of the research.

# 15. REFERENCES

Adler CH, Dugger BN, Hinni ML, Lott DG, Driver-Dunckley E, Hidalgo J, Henry-Watson J, Serrano G, Sue LI, Nagel T Duffy A, Shill HA, Akiyama H, Walker DG, Beach TG. Submandibular gland needle biopsy for the diagnosis of Parkinson's disease. Neurology 2014;82:858-864.

Adler C, Dugger B, Hentz J, Hinni M, Lott D, Driver-Dunckley E, Mehta S, Serrano G, Sue L, Duffy A, Walker D, Beach T. Submandibular gland needle biopsy for the diagnosis of early Parkinson's disease. 19th International Congress of Parkinson's Disease and Movement Disorders, Late Breaking Abstract

Adler CH, Dugger BN, Hentz JG, Hinni ML, Lott DG, Driver-Dunckley E, Mehta S, Serrano G, Sue LI, Duffy A, Intorcia A, Filon J, Pullen J, Walker DG, Beach

TG. Peripheral synucleinopathy in early Parkinson's disease: Submandibular gland needle biopsy findings. Movement Disorders, 2016 Published online DOI:10.1002/mds.26476.

Barbour R, Kling K, Anderson JP, et al. Red blood cells are the major source of alpha synuclein in blood. Neurodegener Dis 2008; 5:55-9.

Beach TG TG, Adler CH, Sue LI, et al. Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. Acta Neuropathol 2010; 199:689-702.

Beach TG, Adler CH, Dugger BN, et al. Submandibular gland biopsy for the diagnosis of Parkinson's disease. J Neuropath Exp Neurol 2011;72:130-6.

Bennett MC. The role of alpha-synuclein in neurodegenerative disease. Pharmacol Ther 2005;105:311-31.

Beyer K, Ariza A. Alpha-synuclein posttranslational modification and alternative splicing as a trigger for neurodegeneration. Mol Neurobiol 2013;47:509-24.

Braak H, Del Tredici K, Rub U, De Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24:197-211

Breydo L, Wu JW, Uversky VN. Alpha-synuclein misfolding and Parkinson's disease. Biochim Biophys Acta 2012;1822:261-85.

Cersosimo MG, Perandones C, Micheli FE, et al. Alpha-synuclein immunoreactivity in minor salivary gland biopsies of Parkinson's disease patients. Mov Disord 2011;26:188-90.

Dabby R, Djaldetti R, Shahmurov M, et al. Skin biopsy for assessment of autonomic denervation in Parkinson's disease. J Neurol Transm 2006; 133:1169:1176.

Del Tredici K, Hawkes CH, Ghebremedhin E, Braak H. Lewy pathology in the submandibular gland of individuals with incidental Lewy body disease and sporadic Parkinson's disease. Acts Neuropath 2010 119(6):703-713.
Devic I, Hwang H, Edgar JS, et al. Salivary alpha-synuclein and DJ-1: potential biomarkers for Parkinson's disease. Brain 2011;134:e178.

Donadio V, Incensi A, Leta V, Giannoccaro MP, Scaglione C, Martinelli P, Capellari S, Avoni P, Baruzzi A, Liguori R. Skin nerve α-synuclein deposits. Neurology 2014;82:1362-1369.

Doppler K, Ebert S, Uceyler N, et al. Cutaneus neuropathy in Parkinson's disease: a window into brain pathology. Acta Neurolopathol 2014 128:99-109.

El-Agnaf OM, Salem SA, Paleologou KE et al. Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. FASEB J 2006;20:419-25.

Hong Z, Shi M, Chung KA et al. DJ-1 and alpha-synuclein in human cerebrospinal fluid as biomarkers of Pakrinson's disease. Brain 2010;133:713-26.

Lebouvier T, Chaumette T, Damier P, et al, Pathological lesions in colonic biopsies during Parkinson's disease. Gut 2008;57:1741-3.

Lebouvier T, Neunlist M, Bruley Des Varannes S, et al. Colonic biopsies to assess the neuropathology of Parkinson's disease and its relationship with symptoms. PLoS ONE 2010;5:e12778.

Lee PH, Lee G, Park HJ, Bang OY, Joo IS, Huh K. The plasma alpha-synuclein levels in patients with Parkinson's disease and multiple systems atrophy. J Neural Transm 2006;113:1435-9.

Li QX, Mok SS, Laughton KM, et a. Plasma alpha-synuclein is decreased in subjects with Parkinson's disease. Exp Neurol 2007;204:583-8.

McLean PJ, Hyman BT. An alternatively spliced form of rodent alpha-synuclein forms intracellular inclusions in vitro: role of the carboxy-terminus in alpha-synuclein aggregation. Neurosci Lett 2002;323:219-23.

Mitchell AW, Luheshi LM, Barker PA. Skin and platelet α-synuclein as peripheral biomarkers of Parkinson's disease. Neurosci Lett 2005;381:294-298.

Mollenhauer B, Locascio JJ, Shulz-Schaeffer W, Sixel-Doring F, Trenkwalder C, Schlossmaker MG. Alpha-synuclein and tau concentrations in cerebrospinal fluid of patients presenting with parkinsonism: a cohort study. Lancet Neurol 2011;10:230-40.

Mollenhauer B, Trautmann E, Taylor P, et al. Total CSF alpha-synuclein is lower in de novo Parkinson patients than in healthy subjects. Neurosci Lett 2-13;532:44-8.

Park MJ, Cheon SM, Bae HR, Kim SH, Kim JW. Elevated levels of alpha-synuclein oligomer in the cerebrospinalfluid of drug neive patients with Parkinson's disease. J Clin Neurol 2011;7:215-22.

Rossi A, Giovenali P, Benvenuti M, Di Iorio W, Calabresi P. Skin biopsy: A new diagnostic tool for autonomic dysfunctions in Parkinson's disease? Lancet Neurol 2007; 6:848-849.

Scherzer CR, Eklund AC, Morse LJ, Liao Z, Locascio JJ, Fefer D, Schwarzschild MA, Schlossmacher MG, Hauser MA, Vance JM, Sudarsky LR, Standaert DG, Growdon JH,

Jensen RV, Gullans SR. Molecular markers of early Parkinson's disease based on gene expression in blood. Proc Natl Acad Sci U S A. 2007 Jan 16;104(3):955-60.

Shannon KM, Keshavarzian A, Mutlu E, et al. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. Mov Disord 2012;27:709-15.

Shi M, Bradner J, Hancock AM, et al. Cerebrospinal fluid biomarkers for Parkinson's disease diagnosis and progression. Ann Neurol 2011;30:6838-51.

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature 1997;388:839-40.

Tokuda T, Salem SA, Allsop D, et al. Decreased alpha-synuclein in cerebrospinal fluid of aged individuals and subjects with Parkinson's disease. Biochem Biophys Res Commun 2006;349:162-6.

Visanji NP, Marras C, Kern DS, Dakheel AA, Gao A, Liu LWC, Lang AE, Hazrati LN. Colon mucosal a-synuclein lacks specificity as a biomarker for Parkinson's disease. Neurology 2015; 84:609-616.

Wang N, Gibbons CH, Lafo J, Freeman R. Alpha-synuclein in cutaneous autonomic nerves. Neurology 2013; 81:1604-1610.

Yanaamandra K, Gruden MA, Casaite V, Meskys R, Forsgren L, Morozova-Roche LA. Alpha-synuclein reactive antibodies as diagnostic biomarkers in blood serum of Parkinson's disease patients. PLoS ONE 2011; 6:e18513.

## APPENDIX 1: SCHEDULE OF EVENTS

| Visit Description | Screening Visit | Biofluid Collection and Skin Biopsy Visit | Colon Biopsy Visit | Submandibular Gland Biopsy Visit |
|---|---|---|---|---|
| Written Informed Consent | X | | | |
| Screening Demographics | X | | | |
| Assign Unique ID | X | | | |
| Review Inclusion/Exclusion Criteria | X | X | X | X |
| Medical History | X | | | |
| Medical History of PD | X | | | |
| Review Family History for PD | X | | | |
| General Neurological Examination | X | | | |
| Physical Examination | X | | | |
| ECG | X | | | |
| Vital Signs | X | X[1] | X[1] | X[1] |
| MDS-UPDRS (Part IV will not be done for healthy controls or early PD subjects) | X[2] | | | |
| Hoehn and Yahr (all subjects) | X | | | |
| Schwab and England (PD subjects only) | X | | | |
| SCOPA-AUT (all subjects) | X | | | |
| UPSIT Administration (all subjects) | X | | | |
| Montreal Cognitive Assessment (MOCA) (all subjects) | X | | | |

| | | | | |
|---|---|---|---|---|
| PD Stage Assignment | X | | | |
| Clinical safety Labs | X[3] | | | |
| DaTSCAN SPECT Imaging | X | | | |
| Blood Collection for safety evaluation (Chem-20, CBC, PT/PTT) | X | | | |
| Blood Collection for whole blood, plasma, serum, DNA and RNA | | X[4] | | |
| Saliva Collection | | X[5] | | |
| LP Procedure | | X[6] | | |
| Skin Biopsy Procedure | | X[7] | | |
| Colon Biopsy Procedure | | | X[8] | |
| Submandibular Gland Biopsy Procedure | | | | X[9] |
| Concomitant Medications | X | X | X | X |
| AE Assessment | X[10] | X[10] | X[10] | X[10] |

[1] Vital signs (blood pressure, heart rate, respiratory rate and temperature) will be obtained prior to the study procedure and at the completion of the study procedure for each of the following procedures: DaTSCAN™, LP, skin biopsy, colon biopsy and submandibular gland biopsy.

[2] MDS-UPDRS can be done at any visit

[3] Clinical safety labs will include Chem-20, CBC, PT, and PTT.

[4] Blood collection (approximately 46 mL) will occur preferably in the fasting state. If fasting is not possible, the subject may have a low fat/low lipid meal. Samples will be processed using procedures in the S4 Laboratory Manual.

[5] Approximately 5 ml of saliva will be collected via passive drool and processed using procedures in the S4 Laboratory Manual. Subjects should refrain from food intake, drinking liquids and using oral hygiene products for at least 1 hour prior to saliva collection.

[6] Lumbar puncture will be performed in the morning, preferably in a fasted state. If fasting is not possible, the subject may have a low fat/low lipid meal. Approximately 20 mL of CSF will be collected and processed (details in the S4 Laboratory Manual).

[7]Skin biopsies, using a punch biopsy under local anesthesia, will be obtained in the paravertebral region and, distal thigh. Approximately 4 biopsies will be obtained (2 from each location), processed per the S4 Laboratory Manual.

[8]Colon biopsy will be performed through flexible sigmoidoscopy. Approximately 8 biopsies will be obtained and processed per the S4 Laboratory Manual.

[9]Submandibular gland needle biopsy will be performed using local anesthesia. Up to 4 attempts of biopsies will be obtained and processed per the S4 Laboratory Manual

[10]AEs will be collected at the visits and during follow-up phone calls after each procedure. A phone call will occur at 7 days (±2 days) following DaTSCAN imaging and LP procedures and at 7 days (±2 days) following skin biopsy, colonic biopsy and submandibular gland biopsy.