PROTOCOL

Title: SynOne Pilot Project

Sponsor: Michael J. Fox Foundation for Parkinson's Research

Principal Investigator: Ken Marek, MD

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SynOne Pilot Project Protocol

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1. PURPOSE OF STUDY

The Parkinson Progression Marker Initiative (PPMI) is a broad program consisting of the primary in-clinic study (PPMI Clinical), as well as other complementary initiatives conducted under the program that will contribute to PPMI's overarching goal to identify markers of disease progression for use in clinical trials of therapies to reduce progression of PD disability.

The purpose of this pilot study is to determine concordance between SynOne skin test and cerebrospinal fluid (CSF) alpha synuclein seed amplification assay (asyn SAA).

1.1 Primary Objective:

The primary objective of this study is to determine the sensitivity and specificity of the SynOne skin test for CSF asyn SAA in PPMI participants (Neuronal synuclein disease (NSD)+ and NSD-).

2. STUDY OUTCOMES

At the conclusion of specimen collection for this pilot study, primary analysis will be implemented where estimates and 95% confidence intervals will be obtained for sensitivity and specificity of SynOne skin test for CSF asyn SAA. If the upper limit of the 95% confidence interval for specificity excludes 80% OR the upper limit of the 95% confidence interval for sensitivity excludes 70%, the study will stop for futility reasons and conclude that SynOne would not be sufficiently valid as a future screening tool for PPMI.

Otherwise, the "go" criteria would be met and we will propose a larger study to address the questions of longitudinal change in SynOne, quantification properties, etc.

3. BACKGROUND AND RATIONALE

The CSF asyn SAA is currently the only biomarker of n-asyn that is validated with sufficient rigor to be disease-defining; this assay is positive in >95% of autopsy-confirmed cases of Parkinson's disease (PD) and dementia with Lewy bodies (DLB)[1,2]. Several other promising asyn biomarkers exist. Those that can be measured on specimens that can be obtained easily and non-invasively are of particular interest to enhance feasible assessment of asyn biomarkers in large studies that enroll a diverse range of participants, and eventually in the clinic.

Detection of pathologic asyn in skin biopsies has emerged as a promising biomarker for NSD. Several methods have been reported. The method with the greatest amount of published evidence to date involves double-immunostaining thick cryosection for neuronal elements (PGP 9.5) and phosphorylated aSyn (pSer129) using immunofluorescence. With this method of co-localization of phospho-asyn with the pan-axonal marker PGP 9.5, only intra-axonal synuclein is measured which enhances specificity of results[3,4]. Several groups have reported >80% sensitivity and >90% specificity of this immunohistochemical (IHC) method for clinically diagnosed disease. Differences in distribution of phospho-asyn in skin structures has also consistently been able to distinguish between PD and multiple system atrophy (MSA) in several studies[4-6].

A commercially available version, the SynOne test is available via the company CND Life Sciences. Gibbons et al[7] recently published a large, prospective multicenter study of SynOne which demonstrated that among 233 individuals diagnosed with alpha-synucleinopathies based on clinical diagnostic criteria, 95% had positive cutaneous phospho-syn. 3.3% of controls were positive. The inter-rater reliability for phospho-asyn quantitation and nerve fiber density quantitation was high (>90%). Tolerability profile was excellent, with only 0.5% of the sample experiencing minor complications and no instances of infection or other serious complications.

The results of this study are encouraging. However, a limitation of the study design was a lack of biomarker characterization of the sample. There is a high rate of misdiagnosis (10-20%) for underlying n-asyn among individuals diagnosed with PD based solely on clinical features[8]. Indeed, in the Gibbons et al study, 19% of the patients enrolled in the study had diagnostic uncertainty when reviewed by an expert panel. A separate lab[9] examined cutaneous phospho-asyn and CSF asyn SAA in a sample of clinically diagnosed PD. The study compared skin p-asyn to CSF asyn SAA against clinical diagnosis of synucleinopathy. Notably, of 31 patients in that study with synucleinopathy as assessed by clinical diagnostic criteria, only 24 were SAA+ and 89% were DAT+. Nevertheless, this study provides an important datapoint that p-asyn and CSF SAA were 90% concordant.

Significance

PPMI is interested in applying n-asyn biomarker assessments early on in the screening paradigm of NSD to identify individuals who may be eligible to enroll in clinical trials. Given the findings by Gibbons et al[7], SynOne is a promising tool to this end. However, in order for it to be used for this purpose in PPMI, SynOne requires validation against confirmed CSF n-asyn positivity with CSF asyn SAA, as the only test that has been validated against ultimate gold standard of pathological diagnosis. As a screening tool, $\geq$ 70% sensitivity and at least 80% specificity of SynOne compared to CSF SAA will make SynOne a valuable tool for screening potential PPMI participants at scale.

Justification of using CSF SAA as the sensitivity/ specificity benchmark

CSF asyn SAA is positive in >95% of cases with Lewy body pathology on autopsy[1,2]. Keeping in mind that the future use of SynOne in PPMI will be to *screen* individuals for pathologic asyn, we acknowledge and accept that a small proportion of individuals who screen negative may in fact have CNS asyn pathology[10]. However, since the primary objective here is to assess whether the use of SynOne may be a more efficient, feasible, affordable, and safer screening tool compared to the current use of CSF asyn SAA, it seems reasonable to use the CSF asyn SAA results as the comparison benchmark.

4. STUDY DESIGN

Cross-sectional observational multicenter study to be conducted as PPMI companion study.

5. STUDY POPULATION

This study will include up to 50 participants currently enrolled in PPMI based on SAA status and other inclusion/exclusion.

6. RECRUITMENT METHODS

Up to 50 participants will be recruited, all of whom are already enrolled in PPMI Clinical and may be at various stages of participation and follow-up. PPMI Clinical participants who are potentially eligible will be provided information regarding this companion study and invited to participate. The clinical site staff will be responsible for recruiting participants into this companion study.

7. PARTICIPANT ELIGIBILITY

7.1 Inclusion Criteria

- Willing and able to provide written informed consent
- Enrolled in PPMI clinical study.
- PPMI clinical participants with already-available SAA results.

7.2 Exclusion Criteria

- History of allergic reaction to local anesthesia for skin biopsies.
- Use of anticoagulants (aspirin or clopidogrel alone is allowed).
- Significantly impaired wound healing or history of scarring or keloid formation.

8. OBTAINING INFORMED CONSENT

The procedures and requirements of the study, together with any potential hazards/risks, and the freedom to withdraw from participation in the study at any time, will be explained to each potential participant as part of the consent process. The consent process will take place in a space that allows for privacy and confidentiality and should allow for enough time for the individual to consider participation and ask any questions. Consent will be obtained by the Site Investigator or delegated study staff, as applicable. Each participant will sign the informed consent to document agreement to participate in the study. The signed informed consent might be uploaded to a secure portal for remote monitoring.

It is the responsibility of the Investigator (or as delegated to the person obtaining consent) to make sure that the participant understands what she/he is agreeing to and that written informed consent is obtained before the participant is involved in any protocol-defined procedures. Each participant will be provided a copy of the consent form.

9. PARTICIPANT ID ASSIGNMENT

The PPMI Participant ID number will be used to identify a participant on all study related documentation. Samples will be shipped to CND Life Sciences lab with the participant identifier information (date of birth, sex, etc.) as required by the vendor.

10. STUDY PROCEDURES

Once consent is obtained, and eligibility is confirmed by the Site Investigator, the participant may be enrolled into the study. Participants will have 3mm punch skin biopsies taken from their upper back, thigh, and knee area. Biopsies will be obtained as per SynOne protocol and using SynOne kit.

If the per-protocol PPMI visit includes a skin biopsy, the per-protocol biopsies can be obtained or not obtained, at the discretion of the participant. Therefore, participants in SynOne pilot study will have 3 skin biopsies collected but may have an additional 2 biopsies obtained for the PPMI clinical protocol, should they choose.

Data Return and Entry

Data will be entered into a separate study database developed in the Indiana University REDCap. REDCap, a secure, web-based, HIPAA compliant, data collection platform with a user management system allowing project owners to grant and control varying levels of access to data collection instruments and data (e.g., read only, de-identified-only data views) for other users. The REDCap database will include fields typically included in the test result form provided by CND. The REDCap database will also include a field for the PPMI participant ID (patno). Test results will be returned from CND to sites. Sites will enter data into REDCap. At study conclusion de-identified data will be transferred to LONI.

11. SAFETY ASSESSMENTS

Skin Biopsy

Risks associated with performing punch biopsies of the skin include pain and bruising at the site where the biopsy is taken. There is a small risk that the biopsy site may change color. The skin biopsy may leave a scar. There is also a small possibility of infection or bleeding at the biopsy site. Although very rare, it is possible to have an allergic reaction to the local anesthetic (lidocaine) or betadine.

All applicable safety assessments will be completed in the REDCap system. Information collected from those assessments will be combined with any additional information collected for this protocol.

12. CONCOMITANT MEDICATIONS

Participants receiving anticoagulants, including but not limited to the following medications, are excluded from this study: Dabigatran (PRADAXA, PRADAX, PRAZAXA), HEPARIN, Warfarin (COUMADIN, JANTOVEN), Lepirudan (REFLUDAN), Bivalirudin (ANGIOMAX), and Desirudin (LPRIVASK). Other concomitant medications, including over the counter (OTC), dietary supplements (e.g., herbal remedies) or prescriptions, are permitted except as restricted by the PPMI Clinical protocol. All concomitant medications reported (per instruction in PPMI Clinical Assessments Manual) at the time of SynOne skin biopsy visit are recorded on the study medication log in the PPMI database.

13. RISKS TO PARTICIPANTS

Risks of skin biopsy include pain and bruising at the site where the biopsy is taken. There is a small risk that the biopsy site may change color. The skin biopsy may leave a scar. There is also a small possibility of infection or bleeding at the biopsy site. Although very rare, it is possible to have an allergic reaction to the local anesthetic (lidocaine) or the disinfectant.

Loss of Confidentiality: Participation in this research carries a risk of loss of confidentiality, related to clinical diagnosis, genetic information, among others. It may not be in the best interest of the participant for insurance companies or their employer to become aware of such information. Every effort in compliance with applicable laws will be made to maintain confidential information and protect personal information obtained as a result of this study.

There may be risks or side effects which are unknown at this time.

14. POTENTIAL BENEFITS TO PARTICIPANTS

There are no direct anticipated benefits to study participants in this study. However, new information may be generated by the study that will support development of better treatments for Parkinson's disease.

Participants will be provided with the results of the skin biopsy if they choose. CND will send the report of the results to the site team and the site team will provide the results to the participant.

15. COSTS FOR PARTICIPATION

All research travel, assessments and tests will be provided with no cost to the study participant.

16. PAYMENT AND REIMBURSEMENT FOR PARTICIPATION

Participants will be remunerated for the completed study visits. Participants who require travel to the clinical site, or incur other costs associated with a study visit, will be reimbursed according to the study reimbursement guidelines. Participants will receive funds according to the same method of payment as they have chosen under the PPMI Clinical protocol.

17. PARTICPANT WITHDRAWALS

Study participants will be informed during the consent process that they have the right to withdraw from the study at any time without prejudice and may be withdrawn at the Investigator's or Sponsor's discretion at any time. Any information that has already been collected prior to the study participant's withdrawal will not be removed.

18. ADVERSE EVENTS

18.1 Adverse Event Reporting Requirements

Site investigators and coordinators will be instructed to assess for adverse events at the study visit when skin biopsy is conducted, as well as by telephone 2 to 3 [business/working] days following. Adverse experiences, whether observed by the investigator, or elicited from or volunteered by the participant, should be recorded in the study REDCap database. Events occurring outside of the study procedure adverse event reporting period defined above do not require documentation for study purposes (i.e., will not be listed in REDCap).

Any adverse event ongoing at the 2 to 3 [business/working] day reporting telephone visit, should be followed until resolution or stabilization. Adverse events reported following a premature withdrawal or conclusion of participation visit should be followed not more than 30 days from study procedure.

Adverse events will be reported by the site as required by the site's Institutional Review Board (IRB)/Ethics Board.

18.2 Serious Adverse Event Reporting Requirements

Serious adverse events for skin biopsy occurring up to 3 days following the procedure will be documented in the REDCap database and may result in additional follow up with

the site. The investigator will comply with his/her local Institutional Review Board (IRB)/Ethics Board regarding the reporting of adverse experiences.

18.3 Adverse Event Definitions

Adverse Events (AE)

An AE is any undesirable experience occurring to a participant during study participation, whether or not considered related to the study procedure.

Serious Adverse Event (SAE)

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 investigator, places the participant 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 participant 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, treatment emergent, clinical adverse event is not participant to immediate reporting. For example:

- Admission for treatment of a pre-existing condition not associated with the development of a new adverse event.
- Social admission (e.g., participant 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 adverse event (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

18.4 Assessing Relationship of Adverse Events

The assessment of the relationship of an AE to the skin biopsy is a clinical decision based on all available information at the time the event is being documented. The following definitions of the relationship between the AE (including SAEs) and the study procedure should be considered:

- Unrelated - No possible relationship

The temporal relationship between study procedure and the adverse event onset/course is unreasonable or incompatible, or a causal relationship to study procedure is implausible.

- Unlikely - Not reasonably related, although a causal relationship cannot be ruled out. While the temporal relationship between study procedure and the adverse event onset/course does not preclude causality, there is a clear alternate cause that is more likely to have caused the adverse event than the study procedure.

- Possible - Causal relationship is uncertain
  The temporal relationship between study procedure and the adverse event onset/course is reasonable or unknown, and while other potential causes may not exist, a causal relationship to the study procedure does not appear probable.

- Probable - High degree of certainty for causal relationship
  The temporal relationship between study procedure and the adverse event onset/course is reasonable and other causes have been eliminated or are unlikely.

- Definite - Causal relationship is certain
  The temporal relationship between study procedure and the adverse event onset/course is reasonable and other causes have been eliminated.

18.5 Assessing Intensity/Severity of Adverse Events

In addition to assessing the relationship of the adverse event to the study procedure, an assessment is required of the intensity (severity) of the event. The following classifications should be used:

- *Mild:*
  A mild AE is an AE, usually transient in nature and generally not interfering with normal activities.
- *Moderate:*
  A moderate AE is an AE that is sufficiently discomforting to interfere with normal activities.
- *Severe:*
  A severe AE is an AE that incapacitates the participant and prevents normal activities. Note that a severe event is not necessarily a serious event. Nor must a serious event necessarily be severe.

19. STUDY MONITORING AND SITE MANAGEMENT

The PPMI Steering Committee has the responsibility to monitor all procedures for safety, GCP, and regulatory compliance. The study sites will be managed and overseen in an ongoing manner to verify:

(a) The rights and well-being of human participants are protected.
(b) The reported study data are accurate, complete, and attributable.
(c) The conduct of the study follows the currently approved protocol/amendment(s), GCP, and applicable regulatory requirement(s).

20. PRIVACY AND CONFIDENTIALITY

The privacy of participants will be protected in that each person will have the option to voluntarily choose whether to participate in this study. It is the responsibility of the site Investigator to consider the participant's privacy and confidentiality when completing study visits and related protocol activities.

The Site Investigator must assure that the confidentiality of participants, including their personal identity and personal medical information, will be maintained. U.S. sites have additional confidentiality obligations to study participants under the Health Insurance Portability and Accountability Act (HIPAA). Participants will be identified by participant ID numbers on data forms and other study materials submitted to the Site Management Core (SMC), CND Life Sciences, and REDCap. Participants identifiers (name, date of birth, and sex) will be provided to CND Life Sciences and available in REDCap but will not be transferred to LONI.

The Site Investigator will permit the study monitor or designated SMC representative to review signed informed consent(s) and that portion of the participant's medical record that is directly related to the study (or provide certified copies of source documentation upon request). This shall include all study relevant documentation including participant medical history to verify eligibility, laboratory test result reports, admission/discharge summaries for hospital admissions occurring while the participant is in the study, and autopsy reports for deaths occurring during the study (when available). In addition, electronic document storage will be maintained with the Florence electronic trial master file. Identifiable participant information may be stored within this system, which has been validated and deemed compatible with 21 CFR Part 11 requirements. Only study staff requiring access to related study documentation will have permission to view identifiable information.

21. DATA AND SAMPLE SHARING AND STORAGE FOR FUTURE USE

Data collected for this study will be maintained and stored indefinitely at the study cores on secure, password protected systems. All study information (data and samples, as applicable) will be accessed only by those who require access as pertains to the individual's role on the study. All organizations responsible for data storage and review will observe the highest precautions to ensure data integrity and security.

Data collected for this study may be transferred and shared across participating PPMI Cores for conducting analyses as pertains to the study. All PPMI data will be incorporated into the PPMI database to create a fully harmonized PPMI database.

Data obtained during the conduct of this study will be sent to the Laboratory of Neuro Imaging (LONI) in Los Angeles, California to be stored indefinitely for research purposes. Research data will be made available to researchers to conduct analyses related to PD and other disorders. Researchers will be required to comply with the PPMI data agreement to receive data. All personally identifiable information will be removed before it is shared outside the study.

22. ANALYSIS PLAN

This study will compare sensitivity/specificity of SynOne to the CSF asyn SAA. It will calculate sensitivity and specificity of SynOne test for CSF asyn SAA. Estimates for both sensitivity and specificity, along with two-sided 95% confidence intervals will be provided. % expected for age and sex. Analysis will be repeated utilizing a single cervical area biopsy read out.

23. REFERENCES

1. Rossi M, Candelise N, Baiardi S, et al. Ultrasensitive RT-QuIC assay with high sensitivity and specificity for Lewy body-associated synucleinopathies. *Acta Neuropathol* 2020; **140**(1): 49-62.
2. Bargar C, Wang W, Gunzler SA, et al. Streamlined alpha-synuclein RT-QuIC assay for various biospecimens in Parkinson's disease and dementia with Lewy bodies. *Acta Neuropathol Commun* 2021; **9**(1): 62.
3. Gibbons CH, Freeman R, Bellaire B, Adler CH, Moore D, Levine T. Synuclein-One study: skin biopsy detection of phosphorylated α-synuclein for diagnosis of synucleinopathies. *Biomark Med* 2022; **16**(7): 499-509.
4. Waqar S, Khan H, Zulfiqar SK, Ahmad A. Skin Biopsy as a Diagnostic Tool for Synucleinopathies. *Cureus* 2023; **15**(10): e47179.
5. Doppler K, Ebert S, Uceyler N, et al. Cutaneous neuropathy in Parkinson's disease: a window into brain pathology. *Acta Neuropathologica* 2014; **128**(1): 99-109.
6. Donadio V, Incensi A, Rizzo G, et al. Phosphorylated α-synuclein in skin Schwann cells: a new biomarker for multiple system atrophy. *Brain* 2022.
7. Gibbons CH, Levine T, Adler C, et al. Skin Biopsy Detection of Phosphorylated α-Synuclein in Patients With Synucleinopathies. *Jama* 2024.
8. Rizzo G, Copetti M, Arcuti S, Martino D, Fontana A, Logroscino G. Accuracy of clinical diagnosis of Parkinson disease: A systematic review and meta-analysis. *Neurology* 2016; **86**(6): 566-76.
9. Donadio V, Wang Z, Incensi A, et al. In Vivo Diagnosis of Synucleinopathies: A Comparative Study of Skin Biopsy and RT-QuIC. *Neurology* 2021; **96**(20): e2513-e24.
10. Arnold MR, Coughlin DG, Brumbach BH, et al. α-Synuclein Seed Amplification in CSF and Brain from Patients with Different Brain Distributions of Pathological α-Synuclein in the Context of Co-Pathology and Non-LBD Diagnoses. *Ann Neurol* 2022; **92**(4): 650-62.

24. APPENDIX 1 SynOne Pilot Schedule of Activities

Visit	Sample 1	
Assessment		
Consent Activities		
Documentation of Informed Consent	X	
Informed Consent Tracking Log	X	As Needed
General Activities		
Review Inclusion/Exclusion Criteria	I	
Screen Fail	As Needed	
Conclusion of Study Participation		As Needed
Clinical and Biological Samples		
SynOne Skin Biopsy	X	
Safety and General Health		
#Adverse Events In Clinic Assessment	X	
#Adverse Event Telephone Assessment	X	
Report of Pregnancy	As Needed	

I = Investigator completed assessment

P = Participant completed assessment
X = Investigator or Coordinator completed assessment (or as otherwise delegated)

Adverse events collected only day of and 2-3 business days post skin biopsy per protocol.