STAT-PD: Preventing Levodopa Induced Dyskinesia in Parkinson's disease with HMG-CoA Reductase Inhibitors

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Background and Significance

Parkinson's Disease (PD) and Levodopa Induced Dyskinesia (LID): The enthusiasm that accompanied the invention of levodopa for the treatment of the disabling symptoms of Parkinson disease in the late 1960s, was tempered by realization that abnormal movements resembling chorea (but other hyperkinetic movements too) would eventually develop in most chronically treated patients. The term "levodopa induced dyskinesia" (LID) was eventually coined; and scientific inquiry into one of the most puzzling and difficult to treat complications of therapy for PD has eluded a satisfactory solution.

It is reported that up to 50-80% will develop LID within 5 years of L-dopa treatment[1], 80-90% by 10+ years of treatment[2]. There is variability within patients making it difficult to predict when a patient will develop LID; probable risk factors include earlier age at onset of PD, longer duration of L-dopa treatment, initial levodopa dosage, cumulative levodopa dose exposure, female gender, years of disease duration, disease severity and possibly genetic factors[3,4,5,6,7,8]. Cerebrovascular disease was not associated with motor complications, but was associated with pneumonia in another study(9). [3]. In the beginning, LID expression is mild (often unnoticed by the patient) and becomes higher amplitude over the years, or in other words, it becomes more severe.

Quality of life surveys suggest that mild LID is not disabling, but as LID becomes more severe, LID can deteriorate quality of life [4][5]. In a large European 60 center study of the effect of LID on QoL, LID was shown to be associated with depression and significant reductions on all four components of the Parkinson Disease Questionnaire (PDQ-39), a validated survey of quality of life in PD. For example, the "social functioning" subscore was almost 50% worse in those with the highest LID levels compared to those with the mildest severity [12]. The odds were 2.8-3 times higher of being depressed or anxious if the patient also experienced LID in a study of >400 Chinese PD patients [13]. In another European multicenter study LIDs had detrimental effects on the PDQ-39 dimensions of activities of daily living, cognition, stigma, and bodily discomfort [14]. A recent study determined that increasing scores on the London Handicap Score were determined by LID as well as OFF motor scores [15]. Another insight into the effect of LID on QoL comes from a study of early PD patients. Here, the authors determined that in the early years when LID is mild, it does not affect QoL [16]. This is not surprising, as LID is typically mild/ unobtrusive in the early years, but will worsen in amplitude over time. Likely, more severe LID becomes a determinant of QoL. Veterans with PD scored lower in physical and mental health related quality of life scores than those with angina/coronary heart disease, arthritis, chronic low back pain, congestive heart failure, diabetes, and stroke [19].

Dyskinesias also increase cost of care substantially[6]. As 2% of Veterans are diagnosed with PD, and over 55,000 receive care in the VA system[7], it follows that the problem of dyskinesia is clinically significant and costly, not only in financial terms, but in diminished quality of life. Indeed, Veterans with PD and its attendant problems scored lower in physical and mental health related quality of life scores than those with angina/coronary heart disease, arthritis, chronic low back pain, congestive heart failure, diabetes, and stroke[8].

Current treatments: The only preventive strategies are to delay use of dopaminergic medications, particularly levodopa. Many patients or their treating clinicians fear LID so much that he/she will avoid taking levodopa until they become quite parkinsonian[9]. Once LID is established, the only medication accepted for use (though no drug is approved) to diminish its severity is amantadine[10], or else reduce

dopaminergic medication use, which can again cause worsened parkinsonism. The last alternative is invasive deep brain stimulation surgery; which is costly and not without risk

Underlying Mechanisms of the development of LID: The mechanisms underlying the development and expression of LID are complex; our understanding of the cascading pathway of post-receptor molecular mechanisms triggered by dopamine receptor -1 (D1) pathway stimulation (striatum D1 receptors are occupied by dopamine generated by levodopa administration) in abnormal basal ganglia is still incomplete. However, these new insights have produced new targets for preventive interventions in ways not previously imagined.

Dopamine receptors are metabotropic G-coupled protein receptors classified as D1-like (D1 and D5 subtypes) or D2 like (D2-4 subtypes). LID is closely associated with sensitization of D1 receptors which are linked to the "direct" pathway, a circuit that links the striatum with the output nuclei the globus pallidus interna (GPi) and substantia nigra pars reticulata.

Dopamine cell denervation of the substantia nigra coupled with pharmacologic replacement dopaminergic priming cause functional/anatomical changes in the striatrum. It is generally agreed that LID results from pulsatile stimulation of striatal postsynaptic dopamine receptors (by short-acting levodopa) with subsequent associated downstream changes in gene/protein expression, particularly in the medium spiny neurons (MSN) of the striatum[11].

Sensitized striatal MSNs become persistently hyper-responsive. This is likely the result of the dopamine-depleted state and resultant over-expression of parts of the D1 receptor signaling/transduction pathways coupled with persistent exposure to levodopa; sensitization the result of increased stimulation of adenyl cyclase. This coupling then causes activation of various protein kinases. Stimulation of protein kinase A (PKA) causes phosphorylation of dopamine- and cAMP-regulated phosphoprotein (DARPP-32) which inhibits protein phosphate (PP1). In turn, that suppresses dephosphorylation of several targets of PKA, all part of the cascade towards LID in causing changes in the excitability of striatal MSNs. *Loss of long-term potentiation* and inadequate beneficial depotentiation seems important to the genesis of LID due to the loss of the striatum's ability to eliminate extraneous information and normalize synaptic efficiency.

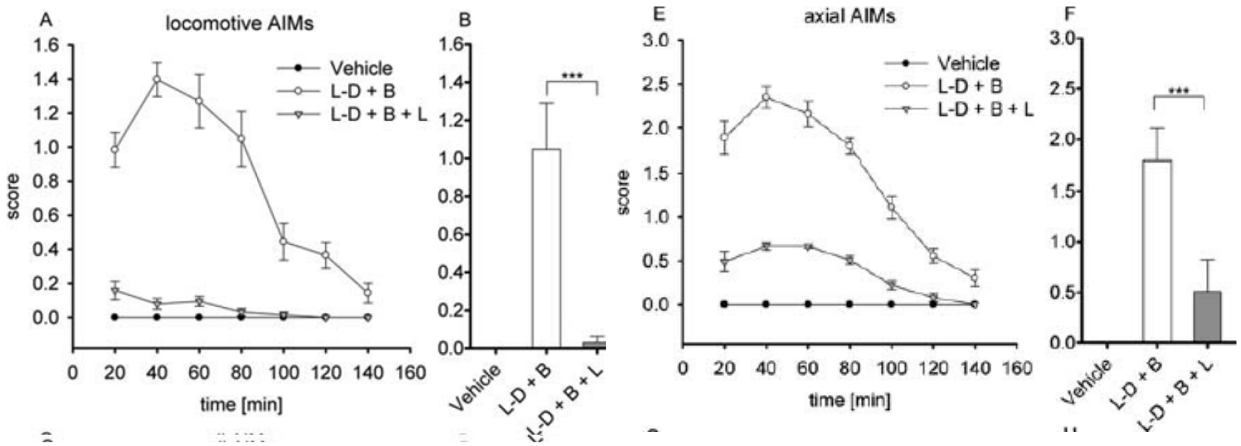
Changes in synaptic plasticity result from alterations in signaling pathways involved in gene regulation and enzyme expression. Extracellular regulated kinases 1 and 2 (ERK1/2), are serine/threonine kinases that are members of the mitogen-activated protein kinase signaling pathway, and are major regulators of transcription/translation as well as *long term potentiation* within the striatum[12]. Hyperphosphorylation and therefore increased activation of ERK1/2 via PKA/DARPP-32 has consistently been associated with LID and expression of several immediate early genes like Nur77, ΔFosB, Zif268 and activity-related cytoskeletal associated protein. This abnormal upregulation ultimately prevents the healthy depotentiation of the striatum perhaps by causing phosphorylation in subunits of the AMPA glutamate receptor (GluA1 subunit)[13]. It has even been proposed that ERK1/2 upregulation may link to the angiogenesis or microvascular remodeling seen in the brains of PD patients and in rats with LID. Inhibition of ERK 1/2 will inhibit this remodeling in the rat model[14]. Other molecular alterations have been described such as increases in protein MSK-1, histone H3 and CREB phosphorylation as well as activation of mammalian target of rapamycin complex 1 (mTORC1), changes which correlate behaviorally in animals with LID expression[15][16].

Pre-clinical evidence for statins to prevent LID development: Given that activation of the Ras-extracellular signal regulated kinase 1/2 (ERK 1/2) is likely associated with development of LID, it follows that blockade of ERK phosphorylation may reduce the expression of LID[17]. Specifically, statins inhibit Ras isoprenylation, therefore can subsequently impede phosphorylation of ERK 1/2 (pERK 1/2)[18].

In a rat model of Parkinsonism and dyskinesia, animals that were pretreated with simvastatin BEFORE levodopa was initiated, compared with vehicle developed 1) less abnormal involuntary movements (AIMS)--which is the animal analogue of dyskinesia, 2) decrease in striatal pERK1/2 3) reduced ΔFosB levels[19].

In another rodent study using lovastatin, the severity of abnormal involuntary movements was clearly attenuated (overall by more than 50%) when the drug was applied BEFORE the initiation of levodopa compared to placebo (see Figure A)[20].

Figure A. Levodopa plus Lovastatin treated animals (L-D + B + L) express significantly less abnormal movements than Levodopa only (L-D + B) treated rodents.



In the MPTP parkinsonian monkey model, simvastatin reduced the severity of established dyskinesia by 45%. Measurement of pERK1/2 in peripheral B and T lymphocytes (CD3/CD20) in these monkeys was significantly different between the vehicle and simvastatin treated animals[21]. These authors also performed an additional intervention with simvastatin in humans with dyskinesia, however the potential problem with the design of this study was that simvastatin was started long after dyskinesia was established and was used short term, so the opportunity to intervene and reduce the priming process had passed.

Table 1. Statin and no Statin patients in PD

Evidence for statin use affecting development of dyskinesia. In a retrospective cohort study by Lieberman et al, PD patients with >7 years of disease had no difference in the composite endpoint of dementia, dyskinesia and wearing off, compared to patients who did not take a statin. However, when examined more closely, dyskinesia appears to be less common on the statin treated group[33]. See Table 1 at right.

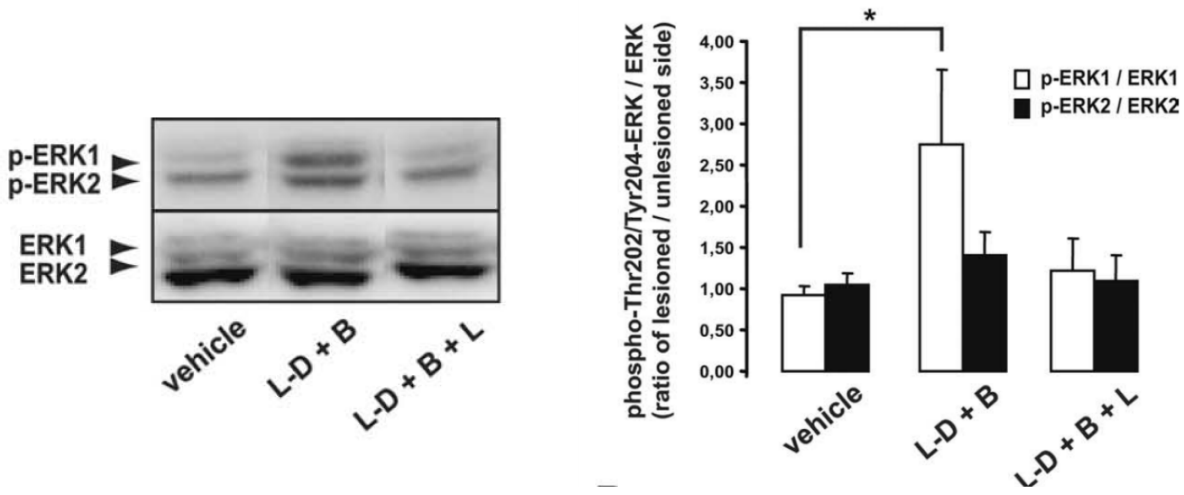
	No Statin	Statin
Patients	200	45
Male/female	123/72 (1.7)[a]	36/9 (4.0)
Age (yrs)	70.6 (11.8)[b]	72.0 (9.4)
Stage (H & Y)[c]	2.6 (1.2)	2.5 (1.2)
PD duration (yrs)	7–22	7–22
Dementia	17 (8.5%)[d]	5 (11.0%)
Dyskinesia	43 (22.0%)	4 (9.0%)
Wearing off	90 (45.0%)	15 (33.0%)

5% Patients on agonist only, 45% patients on sinemet only, 50% patients on sinemet+agonist.

[a] Male/female ratio.
[b] ±Standard deviation.
[c] Stage (Hoehn & Yahr) assessed in 'on'.
[d] Prevalence of dementia, dyskinesia, wearing off.

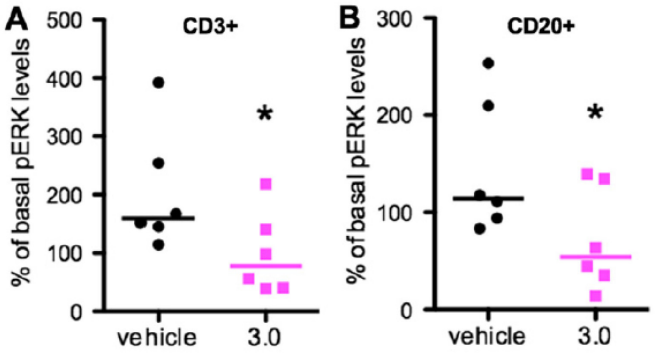
Pre-clinical evidence for ERK1/2 levels as a potential dyskinesia biomarker. In the primary prevention model in which lovastatin was given to the parkinsonian rat before levodopa was commenced, pERK levels in the striatum clearly rose when levodopa alone was administered. This rise was nearly ameliorated with lovastatin. This low pERK correlated with low AIMS (Figure B)

Figure B. West blot demonstrates low pERK in lovastatin (L-D + B + L) treated rodents compared to levodopa only (L-D + B)



In an exploratory trial, Tison and others[22] measured ERK1/2 phosphorylation in both monkeys and PD patients. They collected CD3 and CD20 immunoreactive cells (T and B lymphocytes) after whole blood samples were prepared and incubated with an antibody against phosphorylated forms of Tyr 202 and Tyr 204 of ERK 1/2 using a Becton Dickinson kit. In this study described above, monkeys treated with 3 mg/kg of simvastatin co-administered with LD showed a significant reduction in basal pERK levels (>50% reduction, Figure C). In humans, there was a trend towards reduction in pERK levels that did not reach significance.

Figure C. pERK levels are reduced by 3mg/kg of simvastatin in monkeys.



Summary of Background: Although the biochemical cascading changes that occur in the development of LID are complex, nigral cell loss and chronic LD administration is thought to cause changes in the gene and protein expressions, notably ΔFosB and ERK1/2 amongst many other markers. ERK1/2

hyperphosphorylation in the striatum is implicated in the development of LID as a potential biological molecular marker. Inhibition of ERK1/2 by statins is a putative therapeutic intervention to prevent the biologic cascade leading to dyskinesia. Statin use in rodents and non-human primates with experimental Parkinsonism indicate delay in onset of dyskinesia as well as reduction in the progression of severity over time in LID. While epidemiologic studies of statins and dyskinesia are lacking, data in one study suggested a reduced prevalence of LID in statin-users after 7 years. These studies may also suggest that statin use is optimal when used BEFORE levodopa is begun (priming prevention) rather than secondary prevention, though this remains to be studied further, and there still may be residual benefit to secondary prevention. We can study whether statins can prevent striatum disordered depotentiation and dyskinesia in humans comparing subjects who were prescribed statins for dyslipidemia prior to initiating levodopa (primary prevention) and comparing them to subjects who were not. We can also compare these two cohorts to those who were prescribed statins AFTER starting levodopa to see if there was still a benefit, even if muted (secondary protection). We can test whether pERK levels correlate with each cohort's statin exposure level, as we might expect the lowest levels in those with the longest statin exposure and therefore the least LID.

Preliminary Studies

VA Corporate Data Warehouse Review: We determined we have significant sample to draw upon in VISN 20 to generate our retrospective cohorts. In a preliminary Corporate Data Warehouse, cross-sectional query of patients with PD seen in the PADRECC Movement Disorders Clinic, we discovered 477 living individuals with PD filled and were dispensed both a statin prescription and a LD prescription. Of those, 296 filled a prescription for a statin after starting LD. 181 PD patients filled a prescription for a statin prior to/around the time of an LD prescription fill. The largest group that was generated was the non-statin taking PD subjects filling a levodopa prescription to find matching characteristics from the VISN 20. This was substantial at nearly 900 patients (see Table 2). This information is important given that we will need 40 subjects in each of our 3 cohorts.

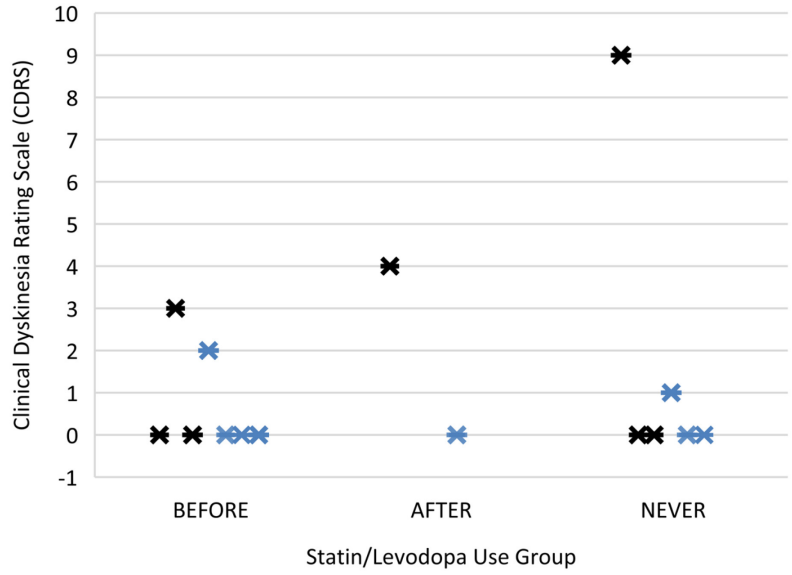
Table 2. Feasibility of Generating Three Cohorts.

Statin prior to Levodopa	Statin after LD	Total PD Patients with both LD and Statin	Non-statin PD patients using LD
181	296	477	900

Clinical pilot data. We obtained pilot data by inviting consecutive PD patients attending a clinic visit to be examined for the presence of dyskinesia. We reviewed by history and by VA pharmacy database records whether statins were prescribed before after or never in relationship to when the patient started L-Dopa. While this "spot" dyskinesia rating is easy to obtain, we confirmed that it is not very accurate compared with performing a levodopa-ON medication cycle (our study protocol involves a levodopa cycle). Out of 19 consecutive data collections, 6 were in levodopa-OFF states (one cannot express dyskinesia if OFF) usually because they forgot to take another dose of medication that day. We discovered that it's not uncommon for patients to take their first dose of medicine in the morning, but then forget to take subsequent doses on a busy day that involves coming to clinic in the afternoon! Of the remaining 13, we found that patient's memory of when they started levodopa and when they started a statin was accurate for most, and we found the pharmacy database to be very accurate and comprehensive.

Results of this small sample may suggest a trend towards more dyskinesia in the never treated group. (see Figure D) Of course, there is no adjustment for confounders like years of levodopa use etc. in this very preliminary dataset.

Figure D. Clinical Pilot Data.



(Legend): dark marks (13) are patients measured in likely ON state (valid), light blue marks (6) are subjects in the OFF or "in between" state (invalid).

Force plate measured of Dyskinesia is Valid, Reliable, and Sensitive. We have a unique method of measuring LID that is precise, reproducible, and sensitive to change. This is due to a combination of techniques. First, since the expression of dyskinesia is dependent on levodopa levels and the ON cycle, we use intravenous IV levodopa to create reliable motor ON cycles. This resource is unique to our center, as the use of IV levodopa for this purpose was pioneered by Director Emeritus John Nutt MD and he continues to hold the IND for IV levodopa (see Rationale for use of Levodopa below).

Second, we have an objective electronic instrument to measure dyskinesia. In our previous work funded by a VA Career Development Award, we determined that a force plate under the feet of a standing subject can quantify the severity of LID when used in subjects who undergo intravenous levodopa administration[23]. We noted that LID caused a unique appearance in the center of pressure (CoP) displacements in PD subjects. Upon inspection of over a dozen parameters quantifying CoP displacements during 30 seconds of stance with a secondary cognitive task, we found that the root mean square of the velocity in the anterior-posterior direction (RMSV) correlated extremely well with clinical LID rating scores (See

Figure E and Figure F).

Figure E. Forceplate CoP plots.

Fig. 1. A Force Plate measures movements of the Center of Pressure (CoP)

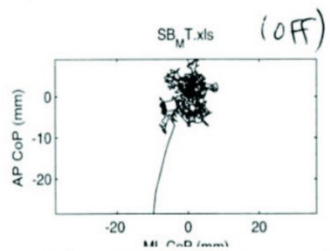


Figure 2.: CoP displacement during stance in a subiect with PD but no LID

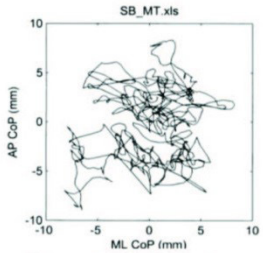


Figure 3: CoP displacement of subject with LID

Figure F. Forceplate Measured Dyskinesia.

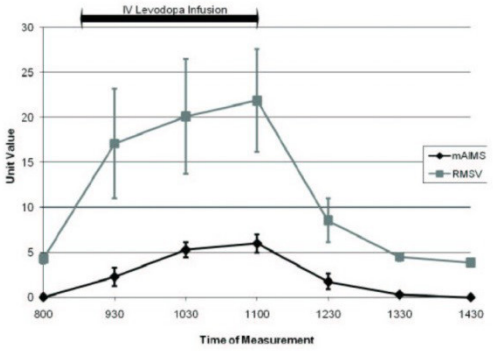


Figure 4. Group means (+/- SEM) of the modified Abnormal Involuntary Movement Scale (mAIMS) and the Root Mean Square of the Velocity (RMSV)—a component of the CoP plot—are parallel.

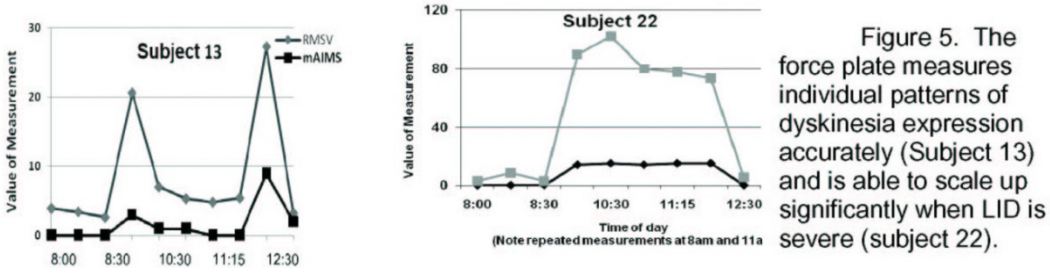


Figure 5. The force plate measures individual patterns of dyskinesia expression accurately (Subject 13) and is able to scale up significantly when LID is severe (subject 22).

These results were developed using clinimetric standards, to measure LID successfully. Not only is the instrument objective because of the nature of instrumentation versus visual (human) ratings, it has proven to be more responsive to detecting changes in LID compared with a visual rating scale. Furthermore, we have shown the force plate to be more reliable under test-retest conditions than visual ratings.

We have demonstrated the effect size measured by the plate to be much larger than a visual rating scale of severity, so that a study utilizing the force plate will require only 41% of the subjects normally needed to demonstrate a change in LID compared with the current gold standard method of visual rating.

Inertial sensor measured LID: iIn the past decade, several attempts to establish an objective measurement of dyskiensia using accelerometers have been undertaken. Many of the studies have

been limited when distinguishing voluntary versus involuntary movement. A neural network approach (multiiple sensors around the body), lend ot richer data on movement, most affected wrist, right, and left ankle. We will use these to measure gait characteristics, dyskinesia, tremor, and attempt to calculate "ON" and "OFF" time. We will use these to measure gait characteristics, dyskinesia, tremor, and attempt to calculate "ON" and "OFF" time. We will use these to measure gait characteristics, dyskinesia, tremor, and attempt to calculate "ON" and "OFF" time

Summary of Preliminary Data: The database pull we secured demonstrates that we have an adequate patient sample base to derivate our three retrospective cohorts. We have sensitive and reliable methods for objectively measuring LID, ensuring that we are actually measuring the outcome we want to study. We have the infrastructure, tools, and the track record of successfully completing clinically relevant and demanding studies. We have a small preliminary prospective trial with a trend that supports the possibility of a statin effect in dyskinesia modulation. A one-look dyskinesia measurement protocol showed that subjects are often in motor states that are invalid for studying dyskinesia expression, confirming the need for an L-dopa cycle to accurately quantify dyskinesia in a standardized way. We are poised to determine if our basic scientists have uncovered a method of intervening with statins to delay the onset and therefore progression of the inevitable worsening in severity of LID.

Specific Aims

Aim 1. Statin Use & LID Development: Determine if statin use coincident (prior) to LD initiation reduced LID development compared with no statin use or late statin use using a retrospective cohort design.

Hypothesis 1: HMG-CoA reductase inhibition by statin interrupts the molecular signaling changes induced by LD in the earliest period of exposure associated with "priming" for LID.

Aim 2 Statin Use & LID Severity: Determine if statin use after LD is initiated reduced LID development (severity) over time compared with no statin use using a retrospective cohort design.

Hypothesis 2: HMG-CoA reductase inhibition by a statin can still be effective in reducing the development of progression of dyskinesia even if started after priming processes are established.

Aim 3 LID and pERK1/2: Determine levels of phosphorylated ERK1/2 levels in CD3/CD20 lymphocytes and compare them with dyskinesia severity.

Hypothesis 3: Dyskinesia severity correlates with phosphorylated ERK1/2 levels and may associate as a dyskinesia biomarker.

Aim 4 LID Measurement: Compare the dyskinesia measurements between the force plate, the inertial sensors, the Unified Dyskinesia Rating Scale (UDysRS), and the Modified Abnormal Involuntary Movement Scale (mAIMS).

Hypothesis 4: The force plate is a valid and reliable method of measuring dyskinesia when compared to the gold standard (mAIMS), a patient/clinician impression of dyskinesia (UDysRS), and a novel form of dyskinesia measurement (the inertial sensors).

Research Design and Methods

Overview: This study is a retrospective three cohort design and will compare statin exposure BEFORE beginning LD, *versus* statin exposure AFTER LD is begun, *versus* NO statin exposure in PD subjects who

are matched for disease characteristics (severity), gender, and total LD exposure. The primary endpoint is the severity of LID between the groups after years of opportunity to develop LID. The power of the VA database is its ability to identify the subjects appropriate for our three cohorts. Dyskinesia amplitude will be measured repeatedly over a single intravenous levodopa cycle, generating an area under the curve (AUC) for each subject, leading to a group AUC for each cohort. All dyskinesia ratings will be by a blinded rater. We will be able to determine whether a statin reduces eventual LID in Veterans who take levodopa for PD, an important public health problem that causes quality of life reduction and raises health care costs for the Veterans Health Administration.

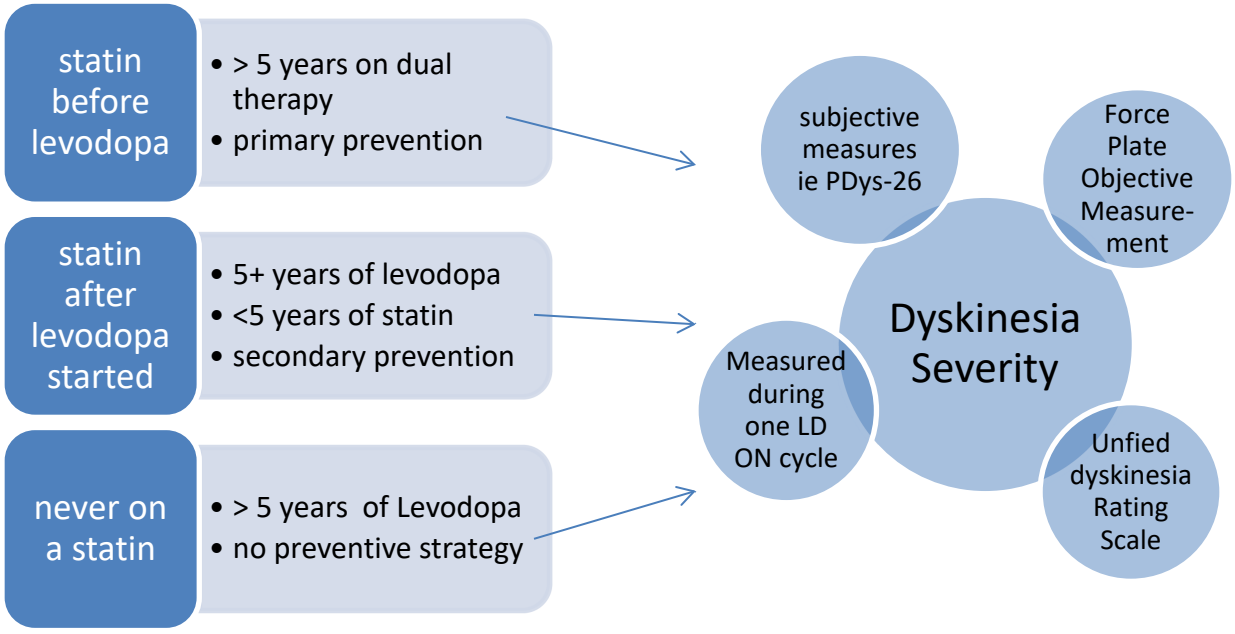
Primary Endpoint: Difference between cohorts in peak Unified Dyskinesia Rating Scale

Secondary Endpoints: Difference between cohorts in AUC of RMSV-AP through levodopa cycle, AUC mAIMS of LID through levodopa cycle

Tertiary Endpoints: QoL scales (PDQ-39, PDys-26,) and ERK1/2 levels as correlated with AUC RMSV – AP through levodopa cycle in subjects

Rationale for study population: We will generate three retrospective cohorts: (Cohort A) Statin Exposure before LD, (Cohort B) Statin Exposure after LD, and (Cohort C) No statin exposure (or LD only). See Figure F.

Figure G. Study Design.



We will also perform a subgroup analysis comparing simvastatin ≤20mg/day (low dose) and a high dose group ≤80 mg/day.

Rationale for Dyskinesia endpoints: We are measuring LID with (A) Unified Dyskinesia Rating Scale, (B) Force Plate, and (C) Subjective Measures.

A. Unified Dyskinesia Rating Scale (UDysRS): The Movement Disorders Society has adopted this scale as the recommended primary measurement tool for dyskinesia. It was determined be best amongst many existing scales to detect changes in dyskinesia using a known treatment that reduces LID severity[24].

B. Force Plate: We have developed a highly *objective* mechanized method of measuring dyskinesia compared to the current gold standard method, which otherwise involves patient *subjective* ratings and observer visual rating scales which are subject to bias and imprecision. (VA Career Development Award)[23].

C. Subjective Measures: Parkinson's Dyskinesia Scale (PDys-26) is a means of assessing the impact of dyskinesia on aspects of everyday living, a quality of life scale[25].

Rationale for the Intravenous Levodopa Cycle: While this method is more intensive for subjects and researchers, it has been one of the best means of studying pharmacokinetics/dynamics of LD and dyskinesia for over 25 years. This system controls many variables that would otherwise widen confidence intervals and require an order of magnitude more subjects to observe significant effects.

The purpose of the IV LD cycle is to control a variable that can alter the moment to moment expression of levodopa-induced dyskinesia (LID)--the degree of the motor ON state the subject is experiencing. The "ON" state is dependent on brain LD levels, which are in turn dependent on plasma LD levels (LD is transported into the brain via a low molecular weight amino acid transporter, competing with other amino acids), thus LID expression is overwhelmingly dependent on LD plasma (and brain) levels. Intestinal absorption of oral LD can be erratic and this variability can introduce unacceptable variability in LID. Secondly, with high protein food intake (in our protocol, protein intake is controlled during the study day), excessive competition for transport of levodopa into the brain can reduce the ON motor state. For these 2 reasons, IV levodopa is a far more controlled way to ensure similar motor conditions that underpin expression of LID.

Rationale for measuring a whole levodopa cycle: Repeated measurements throughout a 6-hour testing period to create an area under the curve (AUC) metric minimizes the impact of erratic outlying single measures. Most importantly though, calculating an AUC after observing a whole levodopa cycle is intuitively superior to single measurements in time, especially when comparing dyskinesia over separate visits. For example, if a one-time LID score was taken and the subject 's levodopa level was not at the peak level of the cycle, yet at the next study visit, the LID score was taken at a good peak levodopa moment, the score could be quite different. This normal variability could overwhelm any effect of an anti-dyskinetic intervention. This is controlled best with an identical dose IV levodopa infusion with repeated dyskinesia measurements during several hours of testing.

1.5 mg/kg/h for 2 hours was chosen for the infusion rate as it consistently produces the motor ON state. This has been established from a 25+ year experience performing infusion studies at our Clinical Translational Research Center. In a review of papers from other centers using LD infusions, no reports of adverse events (AEs) were recorded.[26][27][28] A review of IV LD in PD concluded that this methodology was safe and effective.[29] One report of nausea in subjects receiving IV LD was published by Black et al[30], however in that protocol, subjects received at least 4.5 mg/min loading doses of LD (i.e. over 10 minutes). In IV LD studies at our center, we do not use loading doses, only constant rate infusions. For example, in a 70-kg subject, a 1.5 mg/kg/hr. rate infuses 1.75 mg/minute for the duration of the infusion, a 60% less concentrated amount. Our University site investigator (Dr. J. Nutt) has held an IND for intravenous LD for over 2 decades and one serious AE has been recorded. This serious AE involved overnight hospitalization after a syncopal episode during the second hour of the IV infusion. The IV infusion was immediately discontinued and no sequela occurred. Nausea has rarely been reported by subjects at our center. Based upon nearly 30 years of experience, we have found this method to be easy to administer and easy on subjects. In our pilot study of DHA for prevention of dyskinesia, we have administered over 150 levodopa infusions in the last 4 years, and no serious AEs have occurred.

Methods

Design:

We will utilize a retrospective three cohort design and will compare statin exposure prior to beginning LD, *versus* LD prior to statin exposure *versus* no statin exposure at all, in Parkinson Disease subjects after adjustment for age at onset of PD, duration of L-dopa treatment, initial L-dopa dosage, cumulative dose exposure, female gender, years of disease duration, and disease severity. The main outcome is the severity of LID between the groups after years of opportunity to develop LID. Specifically, each subject's LID is represented by an AUC generated by day testing procedures and the group AUCs generated by each of the three cohorts will be compared.

Setting and Subjects:

We plan to recruit 120 subjects who meet the eligibility criteria (*see below*). The targeted population will be Veterans who are treated at the Northwest Portland Parkinson's Disease Research, Education, and Clinical Center (PADRECC) at the VA Portland Health Care System (VAPORHCS) in Portland, Oregon, the VA Puget Sound Health Care System (VAPUGHCS) in Seattle, Washington, Oregon Health & Science University (OHSU) in Portland, Oregon, and the general public. Given the retrospective nature of this study, we will continue to enroll new subjects until 120 subjects have been recruited. Screening activities will occur at VAPUGHCS or VAPORHCS. All day ratings will occur at Oregon Clinical and Translational Research Institute (OCTRI) located at OHSU unit.

Inclusion of Non-Veterans:

Participants will be recruited from the VA in Portland, the VA in Puget Sound, OHSU, and the general public. Without recruiting from all sites, we would not be able to enroll enough women to make the results generalizable. To obtain a gender representative sample of Parkinson's patients, we will recruit both veterans and non-veterans. According to a meta-analysis, the Parkinson's disease gender ratio 1.49:1 (M:F). The Portland VA's current Parkinson's population contains only 2% female patients, with a gender ration of 39.9:1 (M:F). Since there is a small proportion of females in the VA system (and especially in the Portland VA PD population), recruitment of non-veteran females is necessary to be able to generalize the study to the Parkinson's disease population. For non-veterans, the VHA Notices of Privacy Practices (NOPP) will be either distributed or a link in the consent form will be pointed out during the consenting process. The study team will collect a signed NOPP acknowledgment form (VA form 10-0483) from those subjects and document in the VA CPRS record.

Rationale for inclusion of vulnerable populations:

Not Applicable. Gender, ethnic origin, and minority status are neither inclusion nor exclusion criteria. Women and minorities will be included in this study. Children under the age of 18 will be excluded from this study. No vulnerable populations will be included in this research.

Recruitment:

Subjects will be recruited from PADRECC's (Portland and Puget Sound), and OHSU's Movement Disorders clinics, ResearchMatch.org, FoxTrialFinder.org, a VA Research Contact Repository, an OHSU Research Contact Repository, and from the general public.

VAPORHCS **(Coordinating Site):**

For local recruitment, a partial waiver of informed consent (ICF) process/ authorization (HRA) for screening/recruitment purposes will be obtained for prescreening information (including name, geographical area, date of birth, telephone numbers, social security numbers, medical record numbers, surgical procedures, and diagnostic codes/diagnoses). Clinical recruitment methods are as follows: CPRS charts will be reviewed by the research assistant for PD diagnosis. Charts will be reviewed prior to clinical appointment reminder phone calls at the Portland VA which occur approximately 2 weeks prior to a clinical appointment). Information will be entered into the Main Database (See *Main Database* below Page27). The research assistant, principal investigator, or appointed designee will either during the reminder phone call or before/after the clinical appointment approach the potential participant and inquire as to whether they would like to participate in research (See Recruitment Script). If the potential participant agrees, a telephone consent date will be scheduled. Informed consent and HIPAA Authorization will be mailed or emailed to the participant for their review prior to the screening visit. The participants will be consented at the beginning of the virtual visit. For the virtual consent, a waiver of documentation will be obtained to cover the period between completing the consent with the study staff/principal investigator and the receiving the consent by the research staff. The procedures that may occur during this period are the virtual screening visit. The participants will be asked to either mail in the consent and questionnaires. The participant will be instructed that they cannot email the completed forms back to study personnel. For the Puget Sound participants, the OHSU ICF/HRA will be signed prior to the OHSU visit over the phone.

For those that are part of the VA Neurologic Disorders Repository (NDR; MIRB # 3129; PI: Joseph Quinn MD), all subjects included in the repository have consented to being contacted for opportunities for participation in research. A data use agreement (DUA) will be completed with the repository. Initial contact with potential participants will be made by the research staff following the preferred mode of contact from the repository. The research staff, who may also be a part of the NDR. The recruitment letter, ICF/HIPAA, and flyer will be mailed to interested candidates, and recruitment will proceed as above.

For outside recruitment (OHSU/VAPUGHCS/general public at the VAPORHCS), partial waiver of informed consent/ authorization for screening/recruitment purposes will also include the gathering of private identifiable information needed to contact the participant. The referred potential participant will be called using the phone script. At the end of this call, the subject will be enrolled in the VA medical record system. The information used to enroll are name, address, phone number, date of birth, gender, and social security number. This information is the minimum necessary to enroll.

OHSU **(Recruitment Site):**

A waiver of informed consent process/ authorization will be obtained for prescreening information (including name, geographical area, date of birth, telephone numbers, social security numbers, medical record numbers, surgical procedures, and diagnostic codes/diagnoses). Information will be entered into the Recruitment database (see ***Recruitment Database(s)*** page 27). EPIC charts will be reviewed by the research assistant for PD diagnosis/surgery status. Charts will be reviewed prior to clinical appointment; the treating physician will be emailed a flyer to hand to the potential participant with the VAPORHCS research coordinator's phone number on it and/or an EPIC email will be sent (see recruitment – EPIC Email text). Phone conversation(s) will follow the Recruitment Script). If the potential participant agrees, a screening visit will be scheduled. Informed consent and HIPAA Authorization will be mailed to the participant for their review prior to the initial screening study visit or, for the Puget Sound enrollees, over the phone.

For those that are part of the OHSU Research Contact Repository (eIRB # 8049; PI: Joseph Quinn MD), all subjects included in the repository have consented to being contacted for opportunities for participation in research. Initial contact with potential participants will be made by the Repository research coordinator, who may also be the study coordinator of the repository. The recruitment letter, ICF/HIPAA, and flyer will be mailed to interested candidates, and recruitment will proceed as above.

VAPUGHCS **(Recruitment Site):**

VAPUGHCS will obtain a partial waiver of informed consent process/ authorization for screening/recruitment purposes and recruit similarly to VAPORHCS, using the recruitment script. Information will be entered into the Recruitment database (see ***Recruitment Database(s)*** page 27). As delineated in the VAPUGHCS informed consent document and HIPAA Authorization, after the screening visit the VAPUGHCS will send the screening information and the PII to the coordinating site using MS Outlook with PKI encryption.

General Public

ResearchMatch.org, FoxTrialFinder.org, OHSU Research Opportunities page, ClinicalTrials.gov will all be used for recruitment purposes. In addition, newsletter advertisements will be placed in the NW PADRECC's Parkinsonian quarterly newsletter, the OHSU bi-yearly research newsletter, the Parkinson's Resources of Oregon (PRO) quarterly newsletter. A video advertisement and/or powerpoint advertisement will be posted on virtual events, facebook posts, and/or emailed to participants of a program. OHSU staff and resources will be used for those emailed as part of participation of a program. The email addresses will be collected by OHSU staff as part of the registration process for the particular program.

Email.

Email addresses will be collected prior to consent from the potential participant. If the potential participant wishes to be contacted by email, we will send the appointment letter template for email for screening, maps, the VA informed consent and the VA HIPAA by email to the participant using VA AZURE RMS encryption. The study coordinator will repeat the email address back to the participant to ensure the correct email address is gathered/used. The potential participant will be instructed not to reply to the email but to call the coordinator if s/he has any questions. Emails will only be sent prior to appointments (before screening and/or before day-patient visit) rather than in the mail if the participant wishes this to be the mode of communication.

Inclusion and Exclusion:

Inclusion Criteria:

- PD subjects will be evaluated using the UK Brain Bank Criteria (UKPDBB) [31]. The UKPDBB is a widely accepted clinical criteria for the diagnosis of pd. This is an autopsy-correlated set of criteria that differentiates between Parkinsonian-related disorders, Parkinsonism, and true PD. The clinical diagnosis using the UKPDBB has shown good sensitivity (91%), and specificity (98%), and good positive predicative value (99%) when examined with pathological criteria of PD.
- Age diagnosed ≥ 50 years.
- Treatment with levodopa a minimum of 5 years (≥ 5, to allow LID time to develop)
- Statins for cohort A for duration of levodopa treatment
- Statins may be discontinuous for duration of levodopa treatment in Cohort B

Exclusion Criteria:

- Deep Brain stimulation
- Unable to stand for 1 minute intervals, or sensory deficits in the feet
- Significant cognitive impairment as measured by the Montreal Cognitive Assessment score of < 16
- Subjects with unstable medical or psychiatric conditions (including hallucinations).
- History of unstable medical conditions (i.e. active cardiac disease, recent unwellness, surgery etc.)
- Current use of drugs that may affect parkinsonism or dyskinesia: dopamine receptor blocking medications, depakote, lithium, amiodarone, tetrabenazine, metoclopramide, dronabinol, and illicit drugs such as marijuana (THC), cocaine, methamphetamine
- Unwilling to hold amantadine (if taking) for 24 hours prior to the day-patient visit)

Cohort Generation: Recruitment will occur that patients who come to clinic will be approached for interest in participating as they attend in temporal order and fulfill minimal inclusion/exclusion criterion. We will avoid matching as that can introduce bias. Adjustments for differences in baseline characteristics will be made during data analysis including disease duration, levodopa equivalents, age. Gender is likely to be well matched (male) given the nature of the Veteran population.

Given recruitment demands and studying the evidence for excluding certain statin types our conclusion is that to meet a vigorous recruiting goal successfully, the inclusion of all statins would be beneficial, and unlikely to confound the results of our study.

Measurements

Dyskinesia Measurements:

To establish the severity or the duration of LID, two main measurements will be used; 1) the common standard--the modified Abnormal Involuntary Movement Rating Scale (mAIMS), 2) objective force plate measures. As discussed above, the force plate has been found to be a valid and reliable measurement of LID when compared with the current gold standard.

Clinical Dyskinesia Rating Scale (CDRS)[32]**;** [30 seconds]. This is a commonly utilized scale that is completed by an observer who judges the severity of LID (0-4) in 7 body parts (face, neck, trunk, both legs, and both arms) during the finger tapping task, and during the Get-Up and go task (both described below). CDRS ratings occur as the subject performs the cognitive task while standing on the force plate. CDRS ratings are made every half hour during the LD dose cycle by the principal investigator (KC) or co-investigator.

Force plate measurements; [30 seconds]. The force plate (Advanced Mechanical Technology Inc (AMTI) AccuSway^{PLUS}) system measures three forces and three moments involved in stance. These recordings of center-of-pressure (COP) will be collected every half hour and continuing until subjects turn off‖ as determined by tapping and UPDRS. The subjects will stand on the force plate with their feet 20 cm apart on footprint outlines for thirty seconds while completing a verbal cognitive task such as categorical word fluency, sematic word fluency, or serial subtractions. The addition of the verbal cognitive task significantly aggravates the degree of LID present.

LID can range from severely limiting to not at all bothersome to the individual PD patient. To classify whether the LID that the patient is experiencing is affecting their day to day activities, three self-report measures will be used, the UPDRS Part IV, Parkinson's Dyskinesia Rating Scale, and the Unified Dyskinesia Rating Scale..

Unified Parkinson Disease Rating Scale Part IV (UPDRS-IV); [1 minute]. This is the two-item dyskinesia portion of the UPDRS that asks about duration and disability of LID. This measurement subjectively reports the overall impact of LID on the participant by quantifying the portion of the waking day that dyskinesias are present from 0 (none) to 4 (More than 3/4ths of the day). The second question quantifies how disabling the dyskinesias are perceived to be by the participant from 0 (not disabling) to 5 (completely disabling). Higher scores on this section of the UPDRS indicate more severe dyskinesia. The UPDRS-IV will be given to the patient at baseline, and at each visit (a total of 2 times).

Parkinson Dyskinesia Scale (PDys-26) [33]; [2 minutes]. This is a subject completed questionnaire that quantifies the impact of LID in PD in the last week. This questionnaire captures related but not identical constructs as those of the objective LID measurements and will be used as a complementary measurement of LID to the objective devices.

Unified Dyskinesia Rating Scale (UDysRS); [5-10 minutes]**.** The UDysRS [34] combines patient, caregiver, and treating physician perspectives on both historical (Parts 1 & 2) and objective (Part 3 & 4) assessments of dyskinesia and dystonia. The historical portion and the objective ratings are added together to form total score ranging from 0 to 104 with higher scores indicating more severe dyskinesia.

*(a). Part 1 – On –Dyskinesia Impact***.** This portion of the UDysRS is completed by the patient or caregiver. This part of the UDysRS utilizes modified portions of the UPDRS tool (parts I and II) to assess the daily impact of dyskinesia on activities such as chewing, swallowing, speech, eating, dressing, and handwriting. In this section, the patient or caregiver is asked to rate how dyskinesia impacts various activities on a scale of 0 (normal) to 4 (severe) with higher scores (0 to 44) indicating more severe dyskinesia.

*(b). Part 2 – Historical Disability of Off-Dystonia***.** This portion of the UDysRS is a subject or caregiver completed questionnaire assessing the presence and severity of pain associated with off-dystonia on a 0 (normal) to 4 (severe) scale. This portion of the scale ranges from 0 to 12 with higher scores indicating more severe off-dystonia pain.

(c). Part 3 – Objective Impairment of Dyskinesia. This portion of the UDysRS is an objective evaluation of dyskinesia intensity and is based on the mAIMS. The participant is asked to perform several tasks (describe a picture, drink from a cup, put on a coat and button three buttons, rise from a chair and walk 15 feet) during which the principal investigator rates the intensity and disability of dyskinesias present. Seven body parts are each rated (face, neck, right arm, left arm, trunk, right leg, left leg). Dyskinesia intensity is scored on a 0 (No dyskinesia) to 4 (Incapacitating dyskinesia which prohibits some postures/movements). The highest score for each body part across all tasks is used for computation. This portion of the UDysRS ranges from 0 to 28 with higher scores indicating more severe dyskinesia.

(d). Part 4 – Objective Disability. Dyskinesia disability is scored on a 0 (No dyskinesia observed) to 4 (Dyskinesia interferes/prohibits/or precludes the task) while the subject is performing the four tasks named above. This portion of the UDysRS ranges from 0 to 16, with higher scores indicating more disability.

Inertial Sensors [±8 hours]. The OPAL inertial sensor movement monitoring system will be utilized for this study (APDM, Portland, Oregon). The OPAL system consists of six (6) sensors and a charging/docking unit (See Figure H). Participants will be monitored with the wearable sensors for the entire day of testing. Participants will wear 6 four sensors - on their waist, sternum,

both wrists, and both feet. We will use these to measure gait characteristics, dyskinesia, tremor, and attempt to calculate “ON” and “OFF” time.

Figure H. Inertial Sensors (OPALS)

Parkinsonism Measurements

Several measures of Parkinsonism will occur throughout the day patient visit. These measurements show the clinical state of Parkinson‘s over the course of one LD cycle. Tapping is a measure of bradykinesia or slowness and the UPDRS Part III is a measure of motor function including rigidity and postural stability.

Finger Tapping; [1 minute]**.** Finger tapping speed is measured using a device with 2 counters 20 cm apart and a push down bar mechanism. The levers are pushed as rapidly as possible for 1 minute (done every half hour during the visit). Tapping is an established measurement of bradykinesia in PD[32] and therefore, is a good measure of relative —on and —off states for each individual subject’s day patient visit). An individual increase of 10% (above the 08:00 am score) is accepted as the standard indication of the participant achieving the “on” state.

MDS-UPDRS [2 MIN]. The Movement Disorder Society - Unified Parkinson's Disease Rating Scale (MDS-UPDRS) is a comprehensive assessment that measures the burden and extent of Parkinson's disease. The MDS-UPDRS covers 12 areas of functioning and has four main parts.

Part I. Non-motor experiences of daily living. This 13-question subsection of the UPDRS measures the non-motor experience of daily living. The participant and/or caregiver respond to questions assessing the following domains of non-motor symptoms: cognitive impairment, hallucinations & psychosis, depressed mood, anxious mood, apathy, features of dopamine dysregulation syndrome (strong urges that are hard to control), sleep problems, pain, urinary and constipation problems, light headedness, fatigue. All responses on are a 0 (Normal/No impairment) to 4 (Severe) scale. Summed scores range from 0 to Higher values represent more severe impact on daily living.

PART II. Motor experiences of daily living. This portion of the questionnaire is self-administered. Participants rate 13 aspects of speech, drooling, chewing/swallowing, dressing, hygiene, handwriting, hobbies and activities, turning in bed, tremor, getting out of a car or deep chair, walking & balance, and freezing on a 0 (normal no impairment) to 4 (severe always need help/cannot do on own). Total summed scores range from 0 to 52 with higher values indicating more severe disease state and more burden of disease on daily living.

PART III. Motor examination. The revised Unified Parkinson’s Disease Rating Scale (MDS-UPDRS) motor sub-scale (Part III) will be administered by a movement disorder specialist that has undergone the training recommended by the Movement Disorder Society. Part III of the MDS-UPDRS consists of performing a physical exam on the patient and quantifying 18 specific motor deficits (right/left) on a 5-point scale (0 normal, 1 slight, 2 mild, 3 moderate, and 4

severe). Some of the motor areas examined are rest tremor, action tremor, rigidity, slowness, postural stability, and gait. Total summed score range from 0 to 132 with higher values indicating more severe motor involvement.

PART IV. Motor complications. This 6-item subscale measures the motor complications that occur in advanced Parkinson's disease. The subscale explores three areas: (1) dyskinesia (abnormal involuntary movements that can be described as wiggling, twitching, or jerking), (2) "OFF" time (time when the treatment for Parkinson's doesn't work also described as low time, bad time, slow time, or shaking time), and (3) dystonia (painful cramps or spasms). The subscale assesses total percentage of the day as well as the impact and complexity (or predictability) of the complications. Each complication is rated on a 0 (normal: 0% or NOT present) to 4 (severe: >75% of the time) scale. Total summed scores range from 0 to 24 with higher values indicating advanced Parkinson's disease.

Modified Hoehn & Yahr Staging (H&Y)[36][37]; [1 minute]**.** This is an objective staging scale for rating the clinical functioning of Parkinson's disease patients, combining functional deficits (disability) and objective signs (impairment)[59]. This staging scale is commonly used in both research settings and clinical practice. The Hoehn & Yahr is an ordinal scale ranging from 0 (no signs of disease) to 5 (wheelchair bound/bedridden). The modified version of the scale includes increments of 0.5. The HY has shown good inter-rater reliability and, although it is used worldwide, the clinimetric validity has not been established[60].

Schwab & England Activities of Daily Living Scale (SE); [1 minute]**.** The SE is a widely used measure of disability in Parkinson's with moderate to substantial validity and good reliability[61]. estimates a person's ability to perform daily activities with respect to Parkinson's disease specific constructs such as bradykinesia (slow movement), difficulty dressing, and swallowing. The participant or the provider completes this scale yearly. The scale ranges from high functioning (100% - 'completely independent - Able to do all chores without slowness, difficulty or impairment - essentially normal - Unaware of any difficulty') to highly impaired (00% - 'Bedridden. Swallowing, bladder and bowel functions are not working'). Lower percentages indicate a more dependence on others and lower functioning levels.

Timed Up and Go (TUG); [< 30 seconds]**.** This is a test that measures mobility. The TUG is an average time (seconds) of two trials that involve the participant arising from a chair, walking 25 feet turning around, walking back to the chair, and sitting down. Longer duration of time (seconds) indicates more rigidity, a proxy measure for "ON" time in PD. If the participant has an unsafe gait (as judged by the rater/physician), the TUG will be omitted from the testing.

Clinical Rating Scale – Tremor (CRST); [30 seconds]**.** This is a clinical rating scale examining the severity of tremor in all body areas (head, neck, arms, legs, and trunk). The scale rates from 0 (normal) to 4 (can barely perform the task). Higher values on this scale represent a more severe stage of the disease.

New Freezing of Gait Questionnaire (NFoG). [1-2 minutes]. This is a clinician administered questionnaire assessing both clinical aspects of freezing of gait (FOG) and FOG's impact on quality of life. This questionnaire can be combined with a short video (40 seconds) that demonstrates the different types of freezing. Freezing (sometimes also called a motor block) in Parkinson's disease is a sudden, brief inability to start movement or to continue rhythmic, repeated movements, such as finger-tapping, writing, or walking. This questionnaire is comprised of 9 questions. The first question is a screening question for FOG. All other questions are rated on a 0 to 3 or 0 to 4 scale with higher scores indicating more impact on

daily life and/or more severe freezing. Total NFoG score is computed by summing responses to questions 2 - 9 with a range of 0 to 25 with higher scores indicating more severe FOG. The NFoG has been found reliable in a large sample of non-demented Parkinson's disease patients and shows inter-rater reliability with caregivers of Parkinson's patients [62].

Clinical Global Impression Scale-Severity: [1 minute] This is a seven point rating scale that can be used by clinicians as well as patients to assess severity of a patient's illness, particularly in the context of how the severity of illness affects patient function and behavior.

Quality of Life

Parkinson's Disease Questionnaire 39 (PDQ39); [2-5 minutes]**.** The PDQ39 is a 39 item patient completed survey targeting well-being and functioning in PD [63]. This scale address 8 dimensions (mobility, activities of daily living, emotional well-being, stigma, social support, cognitions, communication, and bodily discomfort)[64]. The PDQ39 dimension scores are on a scale of 0 ("Never") to 4 ("Always/Cannot Do"). Scale scores are summed and range from 0 to 100 with 100 being the maximum level of problems. For a single index figure to characterize the impact of Parkinson's disease upon PD patients (PDSI), all 39 items of the PDQ39 can be summed. The PDQ39 and the use of a PDSI have shown adequate reliability and convergent validity[65].

Environmental Exposure(s)

PaGER Environmental Exposures Questionnaire – Short Version (EEQs), [2-5 minutes]. The EEQs is a 15-item questionnaire assessing environmental exposures in Parkinson's disease. Originally developed as part of the NW PADRECC's *"Parkinson's Genetic Research Study (PaGER)".* This questionnaire assesses the life-time exposure to tobacco, non-steroidal anti-inflammatory drugs (NSAIDs), and caffeine. These factors may contribute to the progression of PD overtime [68].

Cognition

Montreal Cognitive Assessment (MoCA); [15-20 minutes]**.** The MoCA is a short cognitive screening test designed to assist health care professionals for the detection of mild cognitive impairment. The MoCA assesses cognitive function across a variety of domains, such as visuospatial /executive functioning, naming (animals), memory, attention, language, abstraction, delayed recall, and orientation. The MoCA is scored on a 0 to 30 scale, with lower numbers indicating more cognitive impairment. A point is added to the MoCA if the subject's education high school or less (12 years or less education). Scores ranging from 26 - 30 are normal, 18 - 26 mild impairment, 10 - 17 moderate impairment, and less than 10 severe cognitive impairment. The MoCA is a well established screening test in Parkinson's disease patients and is able to discriminate between mild cognitive impairment and dementia [66, 67].

Adverse Events (as recommended by the NINDS Common Data Elements for research reporting). To document medical events that occur to a subject once enrolled. AEs are the construct through which the safety of an intervention is recorded and assessed during a study. All AEs, both serious and non-serious, regardless of relationship to the study intervention, will be recorded on the AE case report form (CRF). AE data will be collected from the time the informed consent form is signed through the duration of the clinical investigation. Typical AE descriptors include event start date, severity, relatedness, outcome, and an indication of whether the event is serious. We will use Common Terminology Criteria for Adverse Events (CTCAE). Local non-serious AEs will be recorded on the CRFs and handled at the local site. Serious AEs will be reported within guidelines to the DSMB, FDA, and IRBs.

pERK1/2 Levels. Immunodetection of pERK with antibody against the phosphorylated forms of Tyrosine202 and 204 of ERK1/2 (AlexaFluor488 BD Biosciences) will occur in lymphocytes isolated from whole blood using flow cytometry. Human peripheral blood mononuclear cells (PBMC) will be isolated using a Ficoll-Paque standard protocol. We will stain CD3 (T lymphocytes) and CD20 (B lymphocytes). Data will be expressed as percentage of pERK cells compared with ERK in a fixed volume of cell preparation.

Plasma Levodopa Measurements. As a quality control measure, plasma levodopa levels will be measured at each 11:00 am directly before the levodopa infusion is discontinued. Blood draw will consist of one 6mL lavender top tube that is centrifuged at 3,000 RPG at -4° C for 15 minutes. Plasma will be aliquoted into two 1.5 mL tubes. Plasma samples will be stored at -80C and analyzed using liquid chromatography mass spectrometry at Dr. Koop's laboratory (OHSU). Batch analysis will occur on the first tube (tube 01) after 30 samples have been collected. At the end of the study, the second tube will be destroyed or placed in the *Neurological Disorders Repository (NDR)* per the participant's wishes.

Framingham Risk Score (FRS). [5 min]. The Framingham Risk Score is a gender-specific algorithm used to estimate the 10- year risk of cardiovascular disease (CVD) risk, cerebrovascular events, peripheral artery disease and heart failure.(53) The algorithm uses age, gender, HDL-C levels, total cholesterol levels, systolic blood pressure, smoking status, diabetes status, and family history of CVD in first degree relative before age 60 to calculate 10-year CVD risk. Using the data from the National Heart, Lung, and Blood Institute's Framingham Heart Study based on predominantly Caucasian population in Massachusetts, USA, risk estimates are divided into risk categories of low (0-10% risk), moderate (10-20% risk), and high risk (20% or more).

Procedures:

Subjects will attend one screening visit and one day patient visit within 3 months of the screening visit. Subjects will be admitted to the day patient in research unit for an intravenous LD dose challenge to accurately determine how much LID they express. LID measurements will be repeated over several hours during the testing day to generate their LID AUC (described below, See

Table 3).

Table 3. Study Procedures

			Visit Procedures	
	Study Visit	**Time Interval**	**VAPORHCS**	**VAPUGHCS/OHSU**
In-Person	Screening (Outpatient)	0	Consent Form/HIPAA Authorization, Inclusion/Exclusion Criteria Parkinson's Assessment/Exams MoCA Vital Signs, ECG, Pregnancy Test (if applicable); Past Medical History; Physical Exam Blood draw (HDL-C/FRS)	Consent Form/ HIPAA Authorization, Inclusion/Exclusion Criteria MoCA Neurological Exam Vital Signs, ECG, Pregnancy Test (if applicable)
			2 hours	**1 hour**
Virtual	Consent	-1	Consent Form/HIPAA Authorization	Consent Form/ HIPAA Authorization
	Screening	0	Inclusion/Exclusion Criteria MoCA Past Medical History	Inclusion/Exclusion Criteria MoCA Past Medical History
			20 minutes	**20 minutes**
	@ Home	Visit - 1	Questionnaires (PDQ39, PDys-26, UPDRS I & II, UDysRS 1 & 2, NFoG)	Questionnaires (PDQ39, PDys-26, UPDRS I & II, UDysRS 1 & 2, NFoG)
	Visit Day patient (Portland OHSU OCTRI)	≤ 3 months	**OHSU ICF/HIPAA Phone Consent (2-3 weeks prior)* **Parkinson's Assessment/Exams* **Past Medical History;* **Blood draw (HDL-C/FRS)* Hold all PD meds overnight before testing Pregnancy test (if applicable) IV LD Infusion Inertial Sensors LID Measurements: (Forceplate, mAIMS, UDysRS 3 & 4, PDys-26, UPDRS IV) Parkinson's Measurements (Finger Tapping, UPDRS-III, TUG, CRST) Blood Collection	** italics indicate methods done for VAPUGHCS & OHSU participants at the Portland site*
			6-9 hours	

Screening:

VAPORHCS: After review and signing of the consent form and HIPAA Authorization with the subject, the inclusion/exclusion criteria will be reviewed. The UKBBPD Criteria will be completed. Inquiries about the subject's medical history, past statin and dopamine agonist use, and current medications will be made. Release of information forms will be completed for medication history stored outside of the VA system and/or outside of Care Everywhere. Baseline data collected on each subject will include: demographic information (such as race/ethnicity, handedness, year of diagnosis, etc), Parkinson's exam (UPDRS parts III and IV, Hoehn & Yahr, Swab & England Activities of Daily Living Scale), a short test of thinking and memory (MoCA), and a dyskinesia scale (PDys-26). Current daily dose of levodopa will be obtained. Additional baseline measurements will be gathered including vital signs, height, weight, and an ECG. A urine pregnancy test will be performed for women of childbearing age; the subject will not be able to continue in the study if pregnant. If the participant comes in fasting, a fasting blood draw will occur and be processed for cholesterol levels by the VA Pathology & Lab Department. Participants recruited from the general public and OHSU will be screened at the VAPORHCS. The OHSU consent/authorization form will be signed at this visit for the all-day visit and questionnaires will be mailed to the participant to complete the week of the day-patient visit.

VAPUGHCS: After review and signing of the consent form and HIPAA Authorization with the subject, the inclusion/exclusion criteria will be reviewed. The UKBBPD Criteria will be completed. Parkinson's exam (UPDRS parts III and IV, Hoehn & Yahr, Swab & England Activities of Daily Living Scale), a short test of thinking and memory (MoCA). Current daily dose of levodopa will be obtained. Additional baseline measurements will be gathered including allergies, vital signs, height, weight, and an ECG. A urine pregnancy test will be performed for women of childbearing age; the subject will not be able to continue in the study if pregnant. These procedures will be performed at the VA Puget Sound. The OHSU consent/authorization form and questionniares will be mailed to the participant and phone consent will be obtained

VIRTUAL VISIT: The screening visit may be conducted virtually using VA Video Connect, WebEx, or HIPAA compliant Zoom. After receipt and review of the consent form and HIPAA Authorization by study staff, the screening visit will occur. The inclusion/exclusion criteria will be reviewed. The UKBBPD Criteria will be completed. Inquiries about the subject's medical history, past statin and dopamine agonist use, and current medications will be made. Release of information forms will be completed for medication history stored outside of the VA system and/or outside of Care Everywhere. Baseline data collected on each subject will include: demographic information (such as race/ethnicity, handedness, year of diagnosis, etc), Parkinson's exam (part IV, Hoehn & Yahr (if safe to do so), Swab & England Activities of Daily Living Scale), and a short test of thinking and memory (MoCA). Current daily dose of levodopa will be obtained. The OHSU consent/authorization form will be signed at this visit for the all-day visit and questionnaires will be mailed to the participant to complete the week of the day-patient visit.

Day patient Visits

All subjects from the recruitment sites (VAPORHCS, VAPUGHCS, and OHSU) will be seen for the day-patient visit at OHSU. Subjects will be required to either be driven to OHSU for this visit or to take alternative means of transportation (bus, tram, taxi, Lyft, or Uber). Subjects will be admitted to the OCTRI day patient unit at OHSU to begin procedures off PD medications. OCTRI is a dedicated research space for clinical studies at OHSU. The center is staffed by RNs for studies, provides nursing equipment (electrocardiographs, blood pressure monitors, etc.), contains a core laboratory, and provides bionutrition services. The core laboratory is adjacent to the clinical unit and provides laboratory support

to studies; performing a wide variety of assays and procedures. The bionutrition unit provides for the provision of research meals.

The subjects will hold their anti-parkinsonian medications while continuing to take their other non-Parkinsonian medications as prescribed, no further PD meds are taken until the end of data acquisition. The first data collection will begin the morning at 9:00AM in the practical OFF state. The subject will then be administered 1.5 mg/kg/hr of intravenous LD for 2 hours (9:30 AM-11:30 AM). The subject is also given oral carbidopa 25 mg at 9:00 AM, 11:00 AM and 13:00 PM to prevent nausea and prevent LD peripheral conversion to dopamine. The purpose of the L-dopa infusion is to create a therapeutic cycle or ON period (the ON period is considered the time the subject experiences the benefit of a dose of medication, with lessening of Parkinsonism. This is overwhelmingly also the time that LID is present). The subjective lessening of Parkinsonism symptoms will be verified by the UPDRS scores and the change in tapping scores. The outcome of interest will be how much LID is manifest during the period when LD is therapeutic. The constant rate infusion of LD produces a very reproducible rising concentration of plasma LD and is very different than the erratic absorption of oral LD.

The levodopa is manufactured by Professional Compounding Centers of America (PCCA) of Houston, Texas. The Lloyd Center Pharmacy (Portland, Oregon) will compound and formulate the levodopa into 1mg/ml clear injectable solution in 50 ml or 100 ml vials. The research study staff will order the compounded levodopa directly from the Lloyd Center Pharmacy using a secure fax line. Information disclosed to the Lloyd Center Pharmacy is date of visit, full name, weight, address, allergies, and date of birth. The OHSU Research Pharmacy will obtain, store, compound, and dispense the intravenous levodopa. The VA Research Pharmacy will obtain and dispense oral carbidopa.

Data are collected at 09:00 AM and every 30 minutes from 10:00 AM until 15:00 PM, or until the subject is OFF as determined by tapping and UPDRS scoring (all procedures are described below under Measurements). At the end of testing for the day, the subject will go home with instructions to continue their medication as usual. See day patient dyskinesia measurement study Procedures summarized on next page (**Error! Reference source not found.**). For participants referred from VAPUGHCS and those with virtual screening visits, inquiries about the subject's medical history, past statin and dopamine agonist use, current medications, and a blood draw for cholesterol levels will be performed (if not performed at screening). Release of information forms will be completed for medication history stored outside of the VA system and/or outside of Care Everywhere. Baseline data collected on each subject will include: demographic information (such as race/ethnicity, handedness, year of diagnosis, etc), and a dyskinesia scale (PDys-26).

Table 4. Table of Day-Patient Procedures.

Procedure	Time of Procedure												
	9:00 AM	9:30 AM	10:00 AM	10:30 AM	11:00 AM	11:30 AM	12:00 noon	12:30 PM	1:00 PM	1:30 PM	2:00 PM	2:30 PM	3:00 PM
Heart Monitoring	X	X	X	X	X	X	X						
IV Levodopa		→	→	→	→	→							
Carbidopa Dose	X				X				X				
Meal								X					
Dyskinesia Ratings													
mAIMS/Force plate	X		X	X	X	X	X		X	X	X	X	X
UDysRS 3 & 4	X				X								X○
Finger Tapping	X[3]		X	X	X	X	X		X	X	X	X	X
Get Up and Go (TUG)	X		X	X	X	X	X		X	X	X	X	X
Tremor Rating	X		X	X	X	X	X		X	X	X	X	X
Inertial Sensors	→	→	→	→	→	→	→	→	→	→	→	→	→
Physical Exam													
MDS-UPDRS-III	X						X						X○
Blood Draw (HLD-C)	X[a]												
Blood Draw (pERK ½)							X						
Blood Draw (LD)							X						
Time (minutes):	30	5	25	5	5	10	10		5	5	5	5	25

[a] only for those not screened at VAPORHCS or did not have blood draw at screen or completed the screening visit virtually

[3] will be repeated 3 times with the last instance being recorded

○ Indicates that this procedure will be performed at the last timed rating of the day (15:00/3:00 pm or when the participant turns "OFF")

Data Analysis.

The peak UDysRS total score will be used as the primary outcome measurement for LID as it is the accepted gold standard. We believe a more sensitive, precise, and reliable measure is our method of AUC calculations with IV levodopa, a full motor ON cycle and the use of an electronic measuring instrument (force plate). Therefore, additional outcome measurements will involve the computation of area under the curve for LID measurements of Modified Abnormal Involuntary Movement Scale (mAIMS) and the forceplate root mean square velocity in the anterior-posterior direction will be computed using the trapezoidal rule.

Primary analysis will follow a per protocol framework, using ordinary least-squares (OLS) regression to examine the difference between the groups (statin before LD, LD before statin, LD without statin) on LID outcome measures while correcting for LID related co-variates. Due to the nature of the retrospective selection, care will be taken to statistically account for potential confounders and modifiers on the relationship between LID and statin use. These will include, but not be limited to, age at onset of PD, gender, length of time taking L-dopa, total daily L-dopa dose, duration of disease, disease severity, and the presence of non PD factors related to a statin such as hypertension. In order to evaluate potential influences of secondary, cerebrovascular effects of indicated statin use on LID as an additional exploratory aim, we will also assess both duration (as a continuous linear variable) and dose of simvastatin (low dose ≤20mg/day and a high dose group ≤80 mg/day) on changes in LID. Secondary outcomes will include examination for treatment group differences in changes in Parkinsonism (tapping AUC, UPDRS III, Hoehn and Yahr, Schwab and England, PDQ-39) including pERK1/2 levels following the previously described methods. Should violations of normal regression assumptions be found, either outcome transformations or alternative regression distributions (e.g. negative binomial to account for positive skew) will be applied as necessary. Sub analyses will examine co-morbidity rates between the groups.

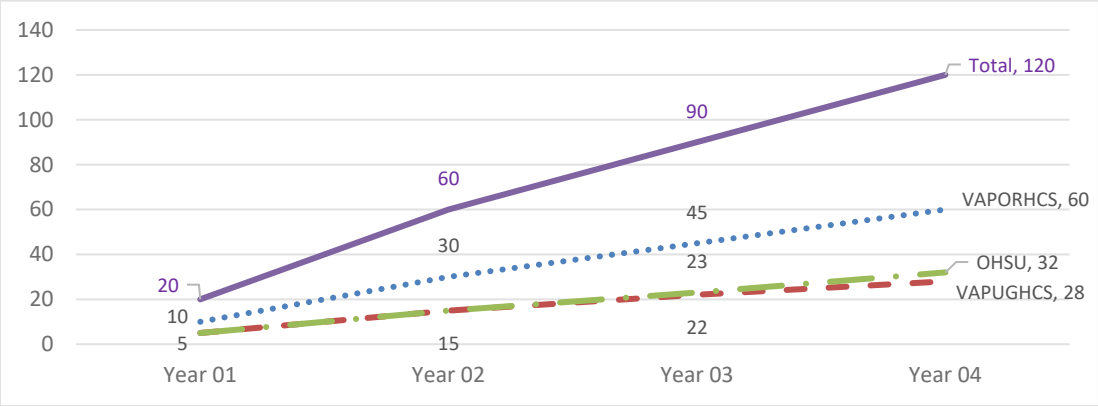
Additionally, pERK1/2 levels will be examined. Initial analysis will examine the relationship between severity of dyskinesia (with the AUC of the RMSV) and pERK1/2 levels using correlations. Further analysis will examine if pERK1/2 levels vary by cohort using ANOVAs and multiple regression techniques. Assessment of confounders of the relationship between pERK1/2 and dyskinesia severity will be examined.

Sample Size: Based on results of a validation study of dyskinesia scales [35], using a one-way ANOVA to examine the difference in part 3, impairment, of the Unified Dyskinesia Rating Scale (UDysRS) between the three pairwise comparisons, 37 participants are needed in each group to detect a moderate effect size (f=0.30, at least one group difference of 3.75 points) with a significance of 0.05 and power of 0.80. Pairwise comparison of statin before levodopa versus no statin will receive priority. To account for drop outs, an additional 3 participants will be included in each of the groups. Additional 10 will be included for screen failures (total 130). Total enrollment will be 40 per group, 120 total. See Table 5 for estimated recruitment goals.

Table 5. Estimated Recruitment Totals per Site

	VAPORHCS	VAPUGHCS	OHSU	Total
Recruitment	60	28	32	120

Figure I. Recruitment Goals over time



Subjects were described previously and in the first cohort will be those who had been on a statin first and remained on it while they were initiated on LD. The second cohort will be those who started a statin AFTER beginning LD. The third cohort will be subjects with no history of statin intake. Matching will occur based on gender, severity of disease based on a PD rating scale, and LD dose

Data Monitoring and Storage:

Main Study Database: The study will generate records of the subjects' medical course. The study will also generate data from the OCTRI. This data will be provided by the subject, and verified by the subject's medical chart, if needed and available. Subject identifiers will be based on the subjects' entry into the study (ex: "STAT-001", "STAT-119"). The Principal Investigator will have access to the code. Data will be entered into a secure password-protected relational database (MS Access) on the VAPORHCS's password-protected network system (located here: \\r01porhsm03\Research\PADRECC\PAD_Chung_STATPD_e17302\Data\). All information is gathered specifically for research purposes. The VAPORHCS will have ownership of the data. All data will be double entered to verify accuracy. The forceplate data will be transformed using MatLab and stored imported into the relational database. Only the study staff and the PI will have access to the database and the forceplate files.

> ***Recruitment Database(s):*** To track recruitment efforts at each location, a mirror of the main database's recruitment portion (tables, queries, forms, reports, etc) of will be created. This mirror will be a unique site-defined password protected MS Access database stored at each location and only accessed by the site. These databases will store PHI, including name, addresses, date of birth, phone numbers, medical record numbers, social security numbers, eligibility status, and recruitment date(s). For OHSU, this database will reside on the X:\ drive in the following location: X:\SOM\Neurology\Parkinson Center\Research & Drug Studies\Active Studies\Chung - #17302 STAT-PD\.

VA Informatics and Computing Infrastructure (VINCI): Data analysis will use the VA Informatics and Computing Infrastructure (VINCI) and the Biostatistician at OHSU. VINCI is a major informatics initiative of the Department of Veterans Affairs (VA) that provides a secure, central analytic platform for performing research and supporting clinical operations activities. It is a partnership between the VA Office of Information Technology (OI&T) and the Veterans Health Administration Office of Research and Development (VHA ORD). VINCI includes a cluster of servers for securely hosting suites of databases

integrated from select national VA data sources. VINCI servers for data, applications and virtual sessions are physically located at the VA Austin Information Technology Center (AITC), located in Austin, Texas. This secure enclave with 105 high-performance servers and 1.5 petabytes of high-speed data storage has multiple layers of security and disaster recovery to prevent data loss.

To ensure the protection of Veteran data, VINCI maintains compliance with the guidelines set forth by Veterans Health Administration (VHA) Handbook 1200.12, Use of Data and Data Repositories in VHA Research, and all other applicable VA and VHA policies and regulations. In addition, VINCI has undergone all security certification activities in support of obtaining an Authorization to Operate (ATO). Access to VINCI resources are approved in accordance with the requirements of National Data Systems (NDS), VHA Handbook 1200.12, Use of Data and Data Repositories in VHA Research, and all other applicable VA and VHA policies and regulations. All data transferred from VINCI is subject to audit for compliance.

VA-credentialed research or operations staff are granted access to study-specific data along with tools for analysis and reporting in the secure, virtual working environment through a certified VHA network computer within the VA. If not working within a VA or VHA hosted office environment containing VA network access, researchers may apply for and then access VINCI through an approved Virtual Private Network (VPN) and Remote Desktop application. The remote computing environment enables data analysis to be performed directly on VINCI servers, offering several advantages: uniform security standards for access; a common point of entry for all investigators who use the data; tools for analysis and reporting; tighter and more consistent control of data quality; and the ability to standardize and update terminology and format as technology and methodology improve.

Storage of Study Data. VINCI will be used only to assist in data analysis; data will not be obtained through VINCI. All study data will be stored in password-protected database stored on the VAPORHCS R01PORHSM03 Server. For data uploaded to VINCI for data analysis, the data will be kept in accordance with the Department of Veterans Affairs Record Control Schedule 10-1 (RCS 10-1). Storage and transfer of any Personally Identifiable Information (PII) or Protected Health Information (PHI) must be done in accordance with applicable VA and VHA policies and directives, state and federal regulations, and applicable statutes including the Health Insurance Portability and Accountability ACT (HIPAA). Unless explicitly requested and approved by data stewards, all sensitive patient data must remain on VINCI project servers and only aggregate data without PII / PHI may be transferred from VINCI. Any desired change in data storage location or transfer requires amending the original data request with an updated of disposition of study data. The amendment must be approved by all data stewards before data may be transferred.

Violations of data policy or approved use of data will be subject to full penalty of law, which may include suspension of access privileges, reprimand, suspension from work, demotion, removal, and criminal and civil penalties.

Upon completion of the research project, the study principal investigator in conjunction with the VA Information Security Officer (ISO), and in accordance with VA policy, will ensure that, study data containing sensitive, confidential information will be returned to the VA, sanitized and removed from all servers, desktops, removable storage devices, etc.

Data Access - VINCI. Only study team personnel explicitly authorized by data stewards will have access to project data. The study principal investigator has the responsibility for security of study. VINCI data managers and VA OI&T personnel not under the purview of the study principal investigator control the servers, network, processors, firewall and software in the VINCI environment, including access rights granted to study personnel.

When study personnel are no longer part of the research team, the study principal investigator will amend the data access request to terminate that person's access to all study data and notify the VA Information Security Officer of such action. No sensitive patient data may be shared with anyone who does not have a VA appointment. All study team personnel with access to sensitive patient data must stay current on required VA information security and privacy policy trainings.

Data Storage Location - VINCI. Study data stored on VINCI servers is located at the Austin Information Technology Center, 1615 Woodward St., Austin, TX 78772-0001. The specific server where the data are stored within the VINCI environment will be chosen by VINCI personnel. The server name and location within the Austin Information Technology Center may be changed at any time at the discretion of VINCI personnel.

Specialized Software. All software used to access sensitive patient data, whether provided by VINCI, or developed by the study team, will run in virtual desktop sessions on VINCI servers within the Austin Information Technology Center.

Electronic CRFs: Puget Sound will receive electronic fillable pdf format case report forms (eCRFs). The form will have a unique RSA encryption key. eCRFs will be printed and completed for each visit manually. Information captured manually will then be entered into the fillable form. eCRFs will be saved with the following file names: Site-Subject-CRFName (examples: PUG-001-Screen, PUG-001-AE). The completed eCRFs will be sent via VA email with PKI encryption to the data coordinator (Lobb) at the VA Portland within 7 days of the visit. The site will receive a confirmation email from the data coordinator who will perform data accuracy and verification checks and enter the information into the Main database described above.

OHSU Statistician. The majority of the analysis will occur at OHSU by Biostatistician, who is listed as a study team member on this study. The dataset will be deidentified. All 18 HIPPA identifiers will be removed from the dataset and age > 90 will be aggregated. Verification of the coded data set creation and the absence of the identifiers will be created and retained in the study files. The research coordinator will be responsible for creating the dataset. The dataset will be password protected and transferred using OHSU'S FIPS compliant secure file transfer system (securex.ohsu.edu).

OHSU FOG Lab: The FOG Lab at OHSU specializes in gait and balance. For analysis coded sensor data will be uploaded to Mobility Exchange (located on OHSU secure server: smb:\\neurodev.ohsu.edu\data); it is a clinical data management system to facilitate the secure collection, storage, and analysis of Mobility Lab (ML) data collected across multiple sites and/or institutions. MX was designed from the bottom up with HIPAA compliance as a requirement, including: 1) Secure login, 2) User based access privileges, 3) Encrypted data uploads over HTTPS/SSL, and 4) Audit trails for added/edited data. An instance of the MX server has been set up at OHSU, in accordance with OHSU's Advanced Computing Center's (ACC) standards for maintenance, backups, and security. Access to this server from outside OHSU's firewall has been established, to enable the collection of data from outside sites. Access to the MX server is only available to users who have been explicitly registered on the server by the server administrator. Once an ML instance has been associated with the MX server, only registered users can use the ML system for data collection and upload their data to MX. All communication between ML and MX is done over an encrypted protocol (HTTPS/SSL). The lab will receive coded, limited dataset that includes inertial sensor data. The lab will receive data after every visit. The research coordinator will be responsible for uploading. The statistician will be added to the OHSU side of the study when assigned by the lab.

Data Repository.

Contact Information: Identifiable data collected under the VA portion of this protocol will be contributed to Dr. Quinn's Recruitment Repository, part of the Neurological Disorders Repository

(MIRB #3129). The NDR Contact Information Source document will be completed. This includes collecting name, telephone number, year of birth, neurological diagnosis, address, and any information the participant would like to volunteer about their study preferences. This data will not be coded, as it is being retained for recruitment purposes. Subjects' consent must be obtained before adding their data to the repository; subjects may decline data banking in this repository and still participate in this study. Data will be kept in \\R01PORHSM03\Services\Research\PADRECC\PAD_Quinn_NDR_3129\Data.

Data and specimens: Post data entry and verification, data and specimens (blood) will be coded and transferred to the *Neurological Disorder Repository* operated by Dr. Joseph Quinn (MIRB # 3129). This repository is maintained at the Portland VA Health Care System. Verification of the coded data set creation and the absence of the identifiers will be created and retained in the study files. The research coordinator will be responsible for the creation and verification process. The key to the code will be maintained by the study and will not be released to the repository. The repository portion of this study is optional.

Data Sharing Plan (IPD):

Individual participant data be available (including data dictionaries). Individual participant data that underlie the results reported the resultant article, after deidentification (text, tables, figures, and appendices). In addition to data, the study protocol and the informed consent form (ICF) will be provided. Data will be available beginning 6 months and ending 2 years following article publication. Data will be shared with Investigators whose proposed use of the data has been approved by an independent review committee ("learned intermediary") identified for this purpose. Data will be released to achieve aims in the approved proposal or for individual participant data meta-analysis. Proposals may be submitted up to 24 months following article publication. After 24 months the data will be available in our VA'S data repository but without investigator support other than deposited metadata. Information regarding submitting proposals and accessing data may be found at (https://www.parkinsons.va.gov/northwest/)

Blood Samples. Blood samples collected during the screening and day-long visit for cardiovascular risk calculation, storage in a repository, levodopa levels (LD), and pERK1/2 analysis. At the screening visit (or at 08:00 am on the day-long visit for the VA Puget Sound participants), approximately 1 mL of blood will be drawn by either the VA phlebotomist or an OCTRI research nurse after 12-18 hours fasting. This blood will be processed by the VA Core lab for a complete metabolic panel. Total cholesterol, HDL-C, systolic blood pressure will be used to calculate the FRS.

At 11:00 am during the day-long visit, approximately 26 mL (5 teaspoons) of whole blood will be drawn. Blood will be placed carried by the research coordinator/assistant directly to the Portland VA where the processing will occur.

Plasma. The samples will be centrifuged to separate plasma. Plasma will be retained (per participant's specimen repository options) in the Neurologic Disorders Repository (NDR; MIRB # 3129). 3mL of plasma will be frozen and stored for levodopa level analysis by the Pharmacokinetics Core at OHSU. Samples will be stored in a freezer located in Dr. Joseph Quinn's Lab at the VAPORHCS. The lab is in building 103 Room E143. Each sample will be coded with the patient's identifier, month and year of collection, and the PI's name and the study number (See Figure J). Samples will be batch processed at the Flow cytometry Shared Resource (FCSR) at OHSU. All batch samples will be hand carried to OHSU by the research coordinator. The flow cytometry core lab at OHSU will provide processing services for the pERK1/2 (extracellular signal-regulated kinase) assays. In particular, the phosho-protein analysis for the

Anti-ERK1/2. Batch analysis of the buffy coat (pERK) and levodopa levels will occur after every 30 samples. The OHSU FCSR has operated as a core resource for OHSU researchers and the Knight Cancer Institute members since 1996 and the Oregon Stem Cell Center since 2005. It provides advanced flow cytometry instrumentation, technical expertise, and technical services. The FCSR provides data analysis, data interpretation, experiment design, and routine operation to researchers.

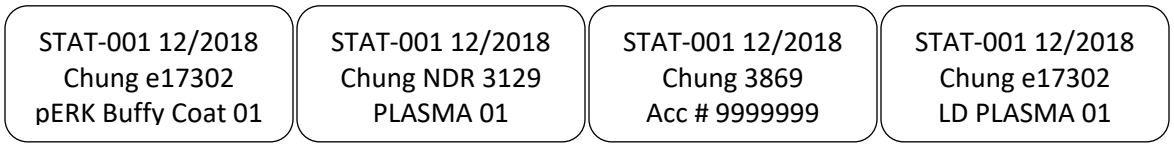


Figure J. Blood tube label examples.

Human Subjects.

Risks to Subjects

Human Subjects Involvement and Characteristics

Involvement of human subjects: Veterans with PD will be asked to attend 1 screening visit at the VAPORHCS, VAPUGHCS, or OHSU and 1 visit in the outpatient unit of OCTRI located at OHSU.

Subject population: 120 subjects veterans and non-veterans will be consented and enrolled. Subjects with probable idiopathic Parkinson Disease, a clinically-based diagnosis based upon history and a physical examination that does not suggest an alternate diagnosis (UK Brain Bank Criterion). Subjects will be diagnosed with PD after the age of 49. Subjects will not be demented, defined by a Montreal Cognitive Assessment of <18, psychotic (hallucinations or delusions by history), or using medications that block dopamine receptors, or have been postulated to worsen parkinsonism or affect dyskinesia. Any unstable medical conditions will also be cause for exclusion. Current substance abuse is an exclusionary criterion. Other classes of PD medications are allowed.

Subject compensation: Participants will be compensated $50.00 for completion of the all-day visit, and $10.00 for the screening visit. Total compensation of $60.00 for completion of the study visits. This compensation is to pay for transportation, food, and potentially time away from work. This compensation is commensurate with previous protocols conducted by the PI involving full-day visits.

Veterans recruited by the Puget Sound site will need to travel to Portland for the day IV levodopa infusion. Travel costs (including Amtrak train from Seattle, Hotel in downtown Portland, and cab fare to and from Oregon Health & Science University) will be reimbursed.

Multi-Site Concerns

VA Research: Research procedures completed at the VA include: recruitment, scheduling/coordination of scheduling, assessment of eligibility, consenting, performing study visit, specimen processing, data entry, study coordination, regulatory tracking/submissions, and data management. VAPORHCS will be the coordinating site.

Non-VA Research: Research procedures completed at OHSU include: recruitment, scheduling/coordination of scheduling, assessment of eligibility, study visit with IV levodopa infusion, blood processing, and data analysis.

Recruitment for this study will occur at the VA Portland Health Care System, the VA Puget Sound Health Care System, and at Oregon Health & Science University. The study staff (PI, site investigators, and research coordinator) are qualified to perform such tasks at both institutions.

Collaborating Sites:

Coordinating Site:

Kathryn Chung, MD — *FWA: IRB00001976*
VA Portland Health Care System (VAPORHCS)
3710 SW US Veterans Hospital Road
Portland, Oregon 97239

Recruitment Site(s):

Cyrus Zabetian, MD — FWA: IRB00007064 or IRB00007065
VA Puget Sound Health Care System (VAPUGHCS)
1660 S Columbian Way
Seattle, Washington 98108

Kathryn Chung, MD — FWA: IRB00000471
Oregon Health & Science University (OHSU)
3181 SW Sam Jackson Park Road
Portland, Oregon 97239

Communication Plan: VAPORHCS is the coordinating center for this study and will oversee the study procedures at both recruitment sites. The Portland sites (VAPORHCS and OHSU) will have one common study coordinator and will use the Joint eIRB Institutional Review Board to ensure that all changes to the protocol, consent form, and HIPAA authorization are communicated. All required approvals will be obtained from each local IRB prior to study initiation. The Portland sites and the Puget Sound site will have a coordinator's conference call once a month to discuss IRB issues, unexpected events (adverse or protocol deviations), and enrollment progress. In addition, quarterly communication between the Portland-PI and the Puget Sound-PI will occur. Site investigators will be informed via email from the principle investigator when effort from their site is no longer required. More frequent communications will occur in the event of adverse events.

Sources of Materials:

The study will generate records of the subjects' medical course, and medications that are taken for the purpose of recording demographic information. The studies will also generate data from the OCTRI. This data will be provided by the subject, and verified by the subject's medical chart, if needed and available. Subject identifiers will be based on the subjects' entry into the study (ex: 001). The Principal Investigator will have access to the code. All information is gathered specifically for research purposes and entered into a secure database on the password-secured network. The data will be kept in accordance with the Department of Veterans Affairs Record Control Schedule 10-1 (RCS 10-1).

Potential Risks:

Levodopa Infusion: Potential risks from the intravenous catheter may include bruising, bleeding, and/or infection. There is a small chance that an infection may occur in the subject's blood or stream or heart valves. The levodopa infusion may cause lightheadedness, low blood pressure, or an irregular heartbeat. Other side effects may include confusion, nausea, vomiting, and an excessive flow of salvia.

Blood Draw: The blood draw could cause pain or bruising at the site of the needle stick. Infrequently people may experience fainting or dizziness. There is a small risk for infection at the site of the needle stick.

Neuroleptic Malignant Syndrome. Withholding Parkinson's medications overnight could result in neuroleptic malignant syndrome. This syndrome is a life-threatening neurological disorder. Symptoms include: high fever, sweating, unstable blood pressure, stupor, muscular rigidity, and autonomic dysfunction.

The probability of adverse events is very low in this study, and if they occur are likely of low seriousness, or transient. Subjects can withdraw from the study at any time. Levodopa infusion studies have been ongoing at our site in Portland for >20 years, Dr. Nutt is the holder of the IND, and one serious AEs has been recorded in this time.

Adequacy of Protection Against Risks

Recruitment and Informed Consent: Subjects will be recruited from the PADRECC Clinic at the VAPORHCS, the Movement disorders clinic at VAPUGHCS, the Movement disorder clinic at OHSU, and the general public. All other enrollment will be subject driven, with listings on ResearchMatch.org, FoxTrialFinder.org, ClinicalTrials.gov, and newsletter advertisements (Parkinson's Disease Research, Education and Clinical Center (PADRECC), Parkinson's Center of Oregon (PCO), and Parkinson's Resources of Oregon (PRO) newsletters), and flyers (posted at the VAPORHCS and OHSU). Patients deemed good candidates will be told of the study and provided with an informed consent form to read at home. If after reading the consent, they are still interested, they will have the opportunity to ask questions about the protocol with the study investigator by phone or at a clinic visit. The consent will be signed by the subject in clinicor by phone. .

Protection against risk: All studies will be reviewed and approved by the VAPORHCS/OHSU Joint IRB and the VAPUGHCS IRB. Study procedures were designed to minimize risk to the subjects. The investigators will adhere to the Data and Safety Monitoring Plan described below.

Data Security: Subjects will be assigned an identification number and this number will be used in the database. Records and data sheets will be kept in a locked file in a locked office in the Parkinson's Disease Research, Education and Clinical Center (PADRECC) area at the VAPORHCS (Building 100 Rooms 7D150A, 7D150C, 7D151). Only the investigators and the research assistant will have access to the coding rubric.

Data Transfer: All data transfers of eCRFs will occur using the VA Outlook email system with PIV encryption. Puget Sound will receive electronic fillable pdf format case report forms (eCRFs). The form will have a unique RSA encryption key. eCRFs will be printed and completed for each visit manually. Information captured manually will then be entered into the fillable form. eCRFs will be saved with the following file names: Site-Subject-CRFName (examples: PUG-001-Screen, PUG-001-AE). The completed eCRFs will be sent via VA email with PKI encryption to the data coordinator (Lobb) at the VA Portland within 7 days of the visit. The site will receive a confirmation email from the data coordinator who will perform data accuracy and verification checks and enter the information into the Main database described above.

For each visit occurring at OHSU, a member of the study staff and/or principal investigator will hand carry the paper chart from the VAPORHCS to OHSU. The chart will remain under control of study staff at all times and will be hand carried back to the Portland VA at the end of the study visit.For analysis of the collected specimen samples, the study coordinator or another member of the study team will hand carry the samples in batches to OHSU for immediate processing.

Drug Safety: Continuous cardiac monitoring will occur during the levodopa infusion and one half hour post infusion. Adverse events (AEs) will be identified by interviewing subjects, examining subjects, vital signs and cardiac rhythm continuous monitoring strips.

Severity of AEs will be graded on a 6-point scale with 0 = none, 1=mild, no treatment needed, 2=moderate, resolved with treatment, 3=severe, required cessation of experimental drug and professional intervention, 4=life threatening and 5=fatal.

Attribution will be on a 5 points scale with definitely related, probably related, possibly related, unlikely to be related and unrelated.

AE will be reported by the PI to the Joint eIRB, and to the FDA using the VA, and DHHS schedule for reporting. The DSMB will review AEs, SAEs, UPs, enrollment, drop-outs, and withdrawals every 6 months. Real time surveillance of AE, dropouts and protocol violations will constitute the safety and efficacy data review. Interim safety review will only take place if 3 or more Serious Adverse Events occur within six months. Otherwise, no other interim safety review is planned and there are no predetermined "stopping rules". If there is a large number of AEs or dropouts, an interim comparison of the prevalence of AEs or measures of efficacy in the two groups can be done without revealing the identification of the groups.

Data and Safety Monitoring Board*:* The DSMB will consist of two movement disorder-trained neurologists (Drs Amie Hiller and Jeff Kraakevik). They will meet in person on a semi-annual basis to review all adverse events and protocol deviations. For serious and other adverse events, the DSMB will have access to unblinded data as necessary. The DSMB chair (Dr. Kraakevik) will generate a written report at each meeting which will be shared with the IRB and PI. If at any point in the study, the DSMB recommends cessation, we will terminate the study. The results of the DSMB will be communicated to the recruitment sites via email.

Potential Benefits of the Proposed Research to the Subjects and Others: In this study, there is no direct benefit to the subjects. Subjects can learn about their disease and how to separate dyskinesia, tremor, "on" "off" knowledge which helps both patient and clinician to manage their disease symptoms more effectively.

Importance of Knowledge to be Gained: The study will contribute to knowledge about whether statins can prevent development and worsening of LID. By developing clinimetric data on the force plate performance, we may also add a device to better study LID particularly in the ambulatory setting. If statins are ineffective, this study will still be very valuable, we will be studying the utility of pERK as a potential biomarker of dyskinesia severity in humans. This knowledge will contribute to understanding how the anti-Parkinsonian response and dyskinesia evolve temporally. The information gathered will also provide clues to future anti-dyskinetic strategies.

Anticipated Results: We expect to demonstrate that subjects on statin prior to initiating LD for PD had lower dyskinesia scores years later when we measured it objectively. Subjectively, we expect these Veterans will have better quality of life and less disability during activities of daily living because of this lessened severity of dyskinesia, as detected by the Pdys-26 and PDQ-39. We also expect that Veterans who initiated a statin after LD will also have reduced eventual dyskinesia compared to those who never took a statin, but not to the same degree as those who started a statin before LD.

We anticipate that pERK1/2 levels measured in peripheral lymphocytes will correlate with dyskinesia AUC as measured by the force plate, an objective method of measuring LID that we developed. We also expect that pERK will correlate with other measurements of dyskinesia such as the Unified dyskinesia rating scale and subjective dyskinesia rating scales, as scores in all these various outcomes do correlate.

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