A phase II, randomized, double-blinded, placebo-controlled trial of liraglutide in Parkinson's disease

INVESTIGATOR-INITIATED STUDY PROPOSAL

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BACKGROUND AND SIGNIFICANCE

Parkinson's disease (PD) is a progressive neurodegenerative condition affecting large numbers of people 65 years and older. Based on prevalence and incidence of the disorder,[1] more than 120,000 people in the U.K.[1,2] and nearly a million people in the U.S.[1,3] suffer from PD with 10,000 new cases in the U.K.[1,4] and 60,000 new cases in the U.S.[1,5] being diagnosed with the condition each year. At present, there is no treatment that can slow or reverse PD progression and all agents currently licensed for the treatment of the disease only temporarily alleviate its motor symptoms.[6-9]

Reduced glucose tolerance in PD is well known and predates introduction of levodopa therapy for the disorder.[10-13] Yet the possibility that this played a role in the pathogenesis of PD was not pursued given several epidemiological reports contending that diabetes was not a risk factor for PD.[14-18] The one epidemiological study on unmedicated diabetics, however, found that diabetes does increase PD risk.[19] This is also the conclusion of the largest epidemiological study on the topic,[20] as well as in several smaller studies of that type.[21-23]

There is reason to believe then that one or more features of diabetes accelerate pathogenesis of PD. One feature likely to do this is systemic insulin resistance, a prominent characteristic of type 2 diabetes (T2D). As noted above, PD cases commonly display impaired glucose tolerance, which is indicative of systemic insulin resistance.[24] That systemic condition can induce brain insulin resistance[25-33] which can also be induced by several early pathogenic factors in PD including α-synuclein,[34] inflammatory cytokines,[35,36] and mitochondrial dysfunction.[37] In this manner, systemic insulin resistance could help drive PD pathogenesis. Indeed, impaired insulin signaling in the brain can cause or exacerbate many brain pathologies and behavioral abnormalities seen in PD, including diminished levels of mitochondrial complexes I-V,[38] increased oxidative stress and apoptosis,[38] elevated striatal dopamine turnover and monoamine oxidase A and B levels,[38] reduced neurogenesis,[39,40] decreased synaptic plasticity,[39,41] impaired cognition,[39,42] anxiety,[38] and depression.[38]

Brain insulin resistance is thus a promising therapeutic target in PD, treatment of which could efficiently slow or stop progression of the disorder. At present, the safest and most reliable means of reducing brain insulin resistance appears to be treatment with receptor agonists of glucagon-like peptide 1 (GLP-1).[43] This is a naturally occurring peptide for which receptors are found throughout much of the brain,[44] including the human cerebral cortex.[45] Two currently marketed GLP-1 receptor agonists with good safety profiles, exenatide and liraglutide, are well known for their neuroprotective effects on animal models of PD.[46-50]

Twice daily administration of exenatide has been tested in a pilot trial on PD patients with encouraging results.[51,52] This single-blind trial on 45 patients showed that those taking the drug for 12 months improved to a significant degree on the Movement Disorders Society's Unified Parkinson's Disease Rating scale (MDS-UPDRS, +2.7 points) compared to a decline (-2.2 points) in control patients not administered the drug. It also showed a highly significant improvement in the Mattis dementia rating scale (MDRS, +2.8 points) in patients on exenatide compared to a decline (-3.5 points) in control patients. A follow-up study a year after the trial ended (i.e., a year off exenatide) found preservation of both the motor and cognitive benefits of the trial.[52] These results suggest a disease modifying effect of the GLP-1 receptor agonist, providing evidence that the pathogenesis of PD can be slowed or stopped. These findings are being pursued in a placebo-controlled, once weekly exenatide (Bydureon) trial on PD cases in London (https://foxtrialfinder.michaeljfox.org/trial/3583/; http://www.ucl.ac.uk/exenatide-pd).

Once daily liraglutide, however, may prove superior to once weekly exenatide in treating PD. While both these GLP-1 receptor agonists can reduce systemic insulin resistance by causing weight loss, once daily liraglutide may be more potent in that regard because it has recently been found more effective than once weekly exenatide in reducing body weight.[53] Liraglutide, which is currently indicated for once-daily administration in adults with T2D up to 1.8 mg/day and for weight management at 3.0 mg/day, is also more effective than exenatide (twice daily or weekly) in reducing hyperglycemia,[53-55] which has diverse detrimental effects on neuronal function.[56-58] Unlike the case for exenatide, there is direct evidence from ex vivo insulin stimulation studies that liraglutide applied directly to brain tissue can reduce insulin resistance there in mild cognitive impairment (MCI) cases in a mouse model of AD.[43] Finally, liraglutide exerts neuroprotective effects in the mouse MPTP model of PD at 25 nmoles/kg body weight, but exenatide does not.[59]

Since it crosses the blood-brain barrier,[60] peripherally administered liraglutide can also reduce brain insulin resistance directly, as verified in studies on a mouse model of AD. Peripheral administration of the drug can have this effect indirectly by promoting weight loss, thereby reducing systemic insulin resistance, which induces brain insulin resistance noted earlier.

Extracellular vesicles (EVs) are membranous particles and are secreted from nearly every cell type throughout the body, whereas the term exosomes refers to a subtype of EVs from 30-150 nm in size that have been implicated in a variety of functions. A recently developed methodology allows isolating plasma EVs enriched for neuronal origin[85]. To date, this approach has been mostly implemented to pursue biomarkers for Alzheimer's disease including the main pathogenic proteins (p-tau and Aβ42) but also intracellular signaling molecules, such as phosphorylated IRS-1, Cathepsin-D, REST, LRP6, and others .[81-84] Of particular interest for the study of brain insulin resistance are the findings concerning IRS-1. In plasma EVs enriched for neuronal origin, total pSer312- and p-PanY- (pan-Tyr phosphorylated) IRS-1 was measured in a clinical cohort of AD patients and cognitively normal (CN) older control subjects (as well as patients with Frontotemporal Dementia, as a neurodegenerative disease control, and cognitively normal patients with diabetes, as a metabolic disease control). Two phospho-species, as well as their ratio, were shown to be highly significantly different in AD patients vs. all control groups. Interestingly, subjects with diabetes had intermediate values between AD patients and CN controls, suggesting that the peripheral IR that characterizes diabetes is linked to some degree to brain IR and corroborating the extensive body of literature suggesting that IR and diabetes are risk factors for AD, but by no means obligatory causative factors. Furthermore, IRS-1 phospho-species achieved remarkable classification accuracy for AD patients vs. controls, and in a separate smaller cohort were already abnormal up to 10 years before clinical onset of AD.[81]

In a recent study, it was shown that a cohort of AD patients without systemic insulin resistance, pSer312-IRS-1 was positively associated with MRI atrophy, whereas p-PanY-IRS-1 was negatively associated with it, in a highly characteristic pattern of regions. The significance of this regional pattern lies in its spatial correlation with the normal IRS-1 brain expression. It is speculated that neuronal-enriched plasma EVs containing IRS-1 may be preferentially derived from brain regions with high levels of IRS-1 expression. Therefore, the IRS-1 phosphorylation pattern seen in these EVs may reflect its phosphorylation status in specific brain regions that suffer brain atrophy in early AD in association with higher burden of brain insulin resistance. These findings provide hope that using these biomarkers may also possibly demonstrate target engagement and follow response to treatment in clinical trials that aim to reverse brain insulin resistance (e.g. liraglutide).

In summary, there is mounting evidence that PD pathogenesis is accelerated by brain insulin resistance and that liraglutide can markedly reduce this abnormality, thereby reducing the motor and non-motor symptoms of the disorder.

SPECIFIC OBJECTIVES:

The primary objective of this clinical trial is to test the therapeutic efficacy and safety of liraglutide treatment of patients with idiopathic PD. The secondary objective is to correlate the magnitude of any observed therapeutic efficacy with degree of reduction in peripheral insulin resistance over the course of drug treatment.

RESEARCH DESIGN AND METHODS:

Study Hypotheses: We hypothesize that global disease status (MDS-UPDRS) and especially cognition in PD patients will improve significantly by the end of daily liraglutide treatment for one year compared to those treated only with the placebo (i.e., drug vehicle). We also hypothesize that the improvement seen in each patient will be proportional to the degree of improvement in peripheral as well as neuronal insulin resistance seen in the treatment period.

Endpoints: The primary outcomes are measures of the drug's effects on motor (assessed by MDS-UPDRS III) and non-motor symptoms of PD (assessed by NMSS and MDRS-2) after 52 weeks of treatment at full tolerated dose. The secondary outcomes are measures of the association between liraglutide's effects on peripheral insulin resistance, markers of insulin resistance on plasma EVs enriched for neuronal origin, PD symptoms and safety.

Study Type: This is a phase II, randomized, double-blinded, placebo-controlled clinical trial.

STUDY POPULATION:

Cases: Fifty-seven patients with a diagnosis of idiopathic PD will be recruited and randomized to receive once daily self-administered injections of liraglutide (1.2 or 1.8 mg, as tolerated) or placebo at the same dose range in a 2:1 study design.

Inclusion Criteria:

- Diagnosis of idiopathic PD according to the United Kingdom Parkinson's Disease Society Brain Bank (UKPDSBB) criteria[a] for at least 2 years, and/or a DaT-confirmed diagnosis of PD
- Responsive to levodopa or dopaminergic treatment
- Male or female between 25 and 85 years of age at time of enrollment
  - Women of child-bearing potential (WOCBP) must agree to use a reliable method of contraception (e.g., oral contraceptive or long-term injectable or implantable hormonal contraceptive, double barrier methods (such as condom plus diaphragm, condom plus spermicide foam, condom plus sponge), or intra-uterine devices) throughout the duration of the trial period and must have a negative serum pregnancy test at screening
  - Male patients with female partners who have child bearing potential must agree to use adequate contraception throughout the duration of the trial period

- Capacity to give informed consent
- Ability to self-administer, or to arrange a care partner to administer trial drug, to comply with trial protocol, and to attend necessary clinic visits off medication

a: Note: the study team will not consider the UKPDSBB criterion "more than one affected relative" as exclusionary. According to the scientific community, family history is not regarded as exclusionary and this specific criterion is no longer used (reference).

Exclusion Criteria:

- Diagnosis or suspicion of other causes for Parkinsonism, including drug- or toxin-induced parkinsonism and other neurodegenerative conditions, including multiple system atrophy, progressive supranuclear palsy, Huntington's disease, Wilson's disease, or Alzheimer's disease
- Active treatment with anticholinergic medications (e.g., triexyphenydil or tricyclic antidepressants)
- Known abnormality on CT or MRI brain imaging considered to cause symptoms or signs of neurological dysfunction, or considered likely to compromise compliance with trial protocol
- Concurrent dementia defined by a score lower than 120 on the MDRS-2 and/or inability to complete scale per neuropsychologist's discretion
- Concurrent severe depression defined by a score greater than 29 on the Beck Depression Inventory (BDI-II)
- Prior intracerebral surgical intervention for PD, including deep brain stimulation, lesional surgery, growth factor administration, gene therapy, or cell transplant
- Already actively participating in a trial of a device, drug, or surgical treatment for PD, or trial participation within 30 days prior to the baseline visit
- Diagnosis of diabetes mellitus of any type, established historically or by:
  - Fasting plasma glucose levels equal or above 126 mg/dl
  - Hemoglobin A1c equal or above 6.5%
- Active treatment with oral antidiabetic medications
- History of severe cardiac disease (e.g., angina, myocardial infarction, or cardiac surgery) in the preceding year
- Significant systemic illness likely to result in deterioration of the patient's condition or, in the Investigator's opinion, affect the patient's safety during the study, including in particular:
  1) History of pancreatitis
  2) Personal or family history of medullary thyroid carcinoma
  3) History of multiple endocrine neoplasia syndrome type 2
  4) History of alcoholism
  5) Severe gastrointestinal disease, including gastroparesis
  6) Treatment with immunosuppressive medications (e.g., systemic corticosteroids) within the last 90 days or chemotherapeutic agents for malignancy within the last 2 years
  7) Severe renal insufficiency (CrCl <30)
  8) Moderate or severe hepatic impairment
  9) Severe hypertriglyceridemia (triglycerides >500 mg/dl)
  10) Body mass index <18.5
- Females who are pregnant or breast feeding
- Prior serious hypersensitivity reaction to Victoza or any of the product components

Withdrawal Criteria: In the absence of a medical contraindication or significant protocol violation,

every effort will be made by the Principal Investigator (PI) to keep accepted subjects in the study. However, should the subject decide to discontinue treatment, all efforts will be made to complete and report the observations as thoroughly as possible, including a complete final evaluation at the time of the subject's withdrawal with an explanation of why the subject is withdrawing from the study. All subjects who prematurely withdraw from the study will receive a follow-up telephone call one month after discontinuation. Study treatment will be stopped if any of the following events occur:

- The subject has any clinical AE, laboratory abnormality, intercurrent illness, or other medical condition that indicates to the PI that continued participation is not in the best interest of the subject or if it requires the subject to stop taking levodopa or other medications essential to the treatment of Parkinson's disease. Subjects experiencing AEs that are present at the end of their participation in the study should receive follow-up, as appropriate. If possible, the outcome of any AE resulting in withdrawal from the study or that was present at the end of the study will be followed until resolution or return to baseline condition, particularly if the AE was considered by the PI to be related to the study drug;
- The subject requires a concomitant medication that is prohibited in the study;
- The subject wishes to withdraw consent from the study in the absence of a medical need to withdraw, as determined by the Investigator;
- The PI concludes that it is in the best interest of the subject to discontinue study treatment;
- The subject is noncompliant; or
- If pregnancy is suspected while the subject is receiving study treatment, the study medication must immediately be withheld until the result of pregnancy testing is known. If pregnancy is confirmed, the study medication will be permanently discontinued and the subject withdrawn from the study. Reasonable attempts will be made to follow the pregnancy to conclusion in order to obtain information regarding the outcome.

The PI must complete all applicable final visit procedures (e.g., safety laboratory parameters, ECG, physical examination, vital signs [including orthostatic BP and HR]) and study documentation for subjects who withdraw from the study treatment and specify the reason for their withdrawal. Once subjects withdraw from the study, they will revert to the care of their usual physician for treatment/management of their disease as appropriate.

Subject Replacement: A subject who withdraws from the study may not reenter the study.

Rationale for Study Population: (see Background and Significance)

Visit Procedures: (see Study Schedule)

Assessments for Efficacy: The efficacy of liraglutide will be assessed by measures of the drug's effects versus placebo on PD motor and non-motor symptoms quantified on the following scales after study medication titration in active and placebo groups:

- MDS-UPDRS part I, II, III and IV changes from ON medication baseline at 28 and 54 weeks
- MDS-UPDRS part III changes from OFF medication baseline at 28 and 54 weeks
- Non-Motor Symptoms Scale (NMSS) changes from ON medication baseline at 28 and 54 weeks

- PDQ-39 Quality of Life scale changes from baseline at 28 and 54 weeks
- MDRS-2 changes from ON medication baseline at 28 and 54 weeks. This test assesses overall cognitive functioning on 5 subscales measuring specific abilities including attention, initiation/perseveration, construction, conceptualization, and memory.
- To complement the MDRS-2, additional neuropsychological assessment tools will include:
  - Delis-Kaplan Executive Function System (DKEFS) Verbal Fluency, a standardized assessment tool with robust psychometric properties. Verbal fluency has been established as a highly sensitive cognitive construct to neuropathology in individuals with PD (e.g., sensitive to striatal pathology or dorsolateral frontal dysfunction).
  - Geriatric Depression Scale
  - The Parkinson's Anxiety Scale.

  Inclusion of these brief mood measures will assist in assessing drug effects on the subjects' health-related quality of life.

Assessments for Safety: Safety and tolerability will be assessed by changes in vital signs, weight, clinical laboratory measures, and adverse events. Routine laboratory studies performed at the beginning and the end of the study will include CBC, renal function, liver function, serum amylase, serum lipase, serum calcitonin[77], and fasting serum glucose and insulin. In addition, an ECG will be performed at baseline and follow-up visits. The number of patients completing the study will be calculated and will establish the tolerability of the study compound at the studied doses. It is expected that over 80% (43 subjects) will safely complete the study.

Other Assessments: Measures of peripheral insulin sensitivity will be used to test the association of changes in such sensitivity to changes in MDS-UPDRS and MDRS-2 scores from baseline to 28 and 54 weeks. Surrogate measures of peripheral insulin sensitivity will be used, specifically the homeostasis model assessment of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI).[61,62] HOMA-IR is defined as fasting glucose (mmol/L) × fasting insulin (mU/L)/22.5. QUICKI is defined as 1/[log fasting insulin (mU/L) + log fasting glucose (mg/dL)].

Note: While the validity of HOMA-IR as a surrogate measure of peripheral insulin sensitivity has been questioned given its failure to correlate with diet-induced insulin sensitivity in dogs[63] and its erroneous prediction of differences in insulin sensitivity between non-diabetic African and European Americans,[64] virtually all clinical studies report a significant correlation of HOMA-IR (or the similarly derived QUICKI index) with insulin sensitivity directly measured with the euglycemic-hyperinsulinemic clamp technique in normal, prediabetic, and diabetic cases of various ethnicities.[65-74] Indeed, the only contrary studies are two reporting that HOMA-IR and/or QUICKI are not correlated with insulin sensitivity in normal Afro-Caribbean adults[75] and a small population of normal Caucasians.[73] Given the body of predictive evidence and to avoid the added expense of oral glucose tolerance tests, the HOMA-IR and QUICKI have been chosen as surrogate measures of peripheral insulin resistance.

In addition, measures of insulin sensitivity in neuronal-origin enriched plasma EVs will be used to test the association of changes in such sensitivity to changes in MDS-UPDRS and MDRS-2 scores from baseline to 28 and 54 weeks. For that purpose, plasma samples will be collected and stored and -80^{o}C to allow for isolation of neuronal origin EVs at the completion of the study.

Subject Compliance: The PI is required to ensure that subjects comply with the drug schedule specified by the study protocol.

Subjects who fail to comply with the drug schedule for 70% of the days since the previous study visit on one occasion will be considered for termination. If the non-compliance was unrelated to an adverse event related to the study drug, then the Investigator will need to evaluate whether the non-compliance is likely to recur. Subjects judged to be high risks for continued non-compliance will terminated at the discretion of the principal investigator. Subjects who fail to comply with the drug schedule for 70% of the days preceding a study visit on a second occasion must be terminated from the study. This mandatory termination due to non-compliance will be reported on the end of study eCRF unless the non-compliance was attributable to adverse effects of study drug.

STATISTICAL CONSIDERATIONS:

Sample Size Calculation: This is a randomized, double-blinded, placebo-controlled clinical trial that aims to evaluate the effects of liraglutide on motor and non-motor symptoms in patients diagnosed with PD. The primary outcome of this study will be the difference in MDRS-2 scores at baseline and follow-up (52 weeks after titration) between the active and placebo control group. The power analysis was performed utilizing data reported in a previous study using a similar GLP-1 agonist (**Table 1**). While score changes associated with active medication were not compared to a placebo group but to a cohort of patients treated with "conventional PD medication", this data represent the closest approximation available for power analysis and sample size calculation.

With a total of 57 patients (38 in the drug group and 19 in the placebo group), the study will have an 82.7% chance of rejecting the null hypothesis of equal means, assuming an average critical difference of 5.1 points on the MDRS-2 and a standard deviation of 6.4 for the drug group and 5.3 for the placebo group with a significance level (alpha) of 0.050 using a two-sided two-sample unequal-variance t-test.

This sample allows for a 12% dropout of up to 6 subjects (e.g., if they are diagnosed with T2D during the course of the study) and still maintain 81.3% power with a total of 51 patients (i.e., 36 cases in the drug group and 15 cases in the placebo group). If the rate of drop out is higher than anticipated, the study will enroll and randomize additional subjects; subjects will be enrolled/randomized in multiples of 3 subjects in order to maintain the 2:1 randomization. The additional enrolled subjects will aim to maintain the power of the study. In total, up to 63 patients will be enrolled and randomized. No more than 42 patients, including drop outs, will be exposed to the drug during the trial.

Statistical Methods: The analysis populations for this study are defined as:
1. Full Analysis Set (FAS): all randomized subjects who receive at least one dose of study drug and have a baseline and at least one post-baseline efficacy score (MDRS-2 and MDS-UPDRS).
2. Per-Protocol (PP) population: a subset of the FAS population, excluding subjects with major deviations from the protocol that may substantially affect the results of the primary efficacy analysis.
3. Safety population: all randomized subjects who receive at least one dose of study drug.
The final determination of protocol violations, and therefore the composition of the PP population, will be made prior to unblinding the database and will be documented separately. Analysis of all efficacy

Table 1 (from Aviles-Olmos et al., 2013[51])

Changes in the score on LED, dyskinesia rating scale, Mattis DRS-2, MADRS, and PDQ39 summary index between baseline and month 14

	Baseline	6 months	12 months	Difference, baseline to 12 months	14 months	Difference, baseline to 14 months
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD); 95% CI	Mean (SD)	Mean (SD); 95% CI
LED						
Exenatide	973 (454)	1011 (518)	997 (446)	24.3 (123.6); −37, 78	1015 (467)	42.3 (137); −22, 107
Conventional PD medication	977 (493)	1061 (613)	1121 (620)	143.7 (223.6); 49, 238	1125 (638)	148.0 (232); 50, 246
Dyskinesia rating scale, on medication						
Exenatide	2.3 (2.8)	2.5 (4.1)	3.3 (4.5)	1.0 (4.2); −1.0, 3.0	3.2 (4.0)	1.0 (3.8); −0.8, 2.7
Conventional PD medication	2.6 (2.9)	3.5 (3.9)	2.5 (2.7)	−0.04 (2.7); −1.2, 1.1	2.7 (3.5)	0.1 (4.3); −1.7, 1.9
MATTIS DRS-2						
Exenatide	140.1 (7.7)	142.4 (2.3)	142.3 (2.5)	2.2 (6.4); −0.8, 5.2	142.9 (2.0)	2.8 (6.0); −0.1, 5.6
Conventional PD medication	139.5 (4.5)	138.2 (5.1)	136.6 (6.1)	−2.8 (5.3); −0.6, −5.0	135.9 (8.5)	−3.5 (5.8); −1.1, −6.0
MADRS						
Exenatide	10.9 (5.1)	8.6 (4.2)	9.0 (4.5)	−1.9 (4.6); −4.1, 0.3	10.2 (6.7)	−0.7 (5.9); −3.5, 2.1
Conventional PD medication	11.0 (5.4)	11.3 (6.5)	11.4 (5.6)	0.5 (4.5); −1.4, 2.4	12.4 (5.6)	1.5 (4.7); −0.5, 3.4
PDQ39 summary index						
Exenatide	19.2 (13.5)	18.1 (13.4)	19.6 (13.0)	0.4 (11.4); −4.9, 5.7	21.5 (19.6)	2.3 (11.6); −3.1, 7.7
Conventional PD medication	24.5 (12.8)	25.2 (15.8)	24.0 (15.3)	−0.6 (11.2); −5.3, 4.2	23.4 (16.0)	−1.2 (9.1); −5.0, 2.7

variables will be performed on the FAS and PP population. The FAS population will serve as the primary population for the analysis of efficacy data. Analyses based on the PP population will be considered as a sensitivity analysis. Subjects will be analyzed for efficacy according to the treatment to which they were randomized.

All safety outcomes will be analyzed using the Safety population. Subjects will be analyzed for safety according to the treatment they actually received.

Descriptive statistics: The study population will be described using demographic, baseline, and subject characteristics. Descriptive statistics of demographic, baseline, and subject characteristics will be based on the Safety population. A detailed description of subject disposition will include:

- A summary of data on subject discontinuations including reason for withdrawal
- A summary of protocol violations and deviations

All screened subjects will be accounted for in the disposition summaries. The number and percentage of subjects included in each analysis population will be summarized.

Baseline and subject characteristics will include a summary of the following:

- Subject demographics
- Medical history including family history

- Physical examination findings
- Prior and concomitant medications/therapies
- HoMA-IR and QUICKI index at baseline
- MDRS-2 scores at baseline
- MDS-UPDRS scores at baseline
- Non-Motor Symptoms Scale (NMSS) scores at baseline
- PDQ-39 Quality of Life scale scores at baseline
- Delis-Kaplan Executive Function System (DKEFS), Geriatric Depression Scale, and Parkinson's Anxiety Scale scores at baseline

Inferential Statistics on Primary Efficacy Parameter(s): The primary analysis of efficacy endpoint (changes in UPDRS, NMSS, and MDRS-2 scores between baseline and Week 54) will be assessed using a two-sided, two-sample unequal-variance t-test to evaluate the differences in end points between the active and placebo cohorts. Multiple regression analysis will be used for comparisons of between-cohort differences in these outcomes over time. General linear models will be used to evaluate possible associations between baseline characteristics and the primary endpoints. Analysis of covariance (ANCOVA) will be used to evaluate possible effects of peripheral insulin sensitivity and treatment group (independent variables) on the primary end points (dependent variable). Hochberg's method for adjustment for multiple testing separately for the primary end points and secondary endpoints will also be used.

The last UPDRS, NMSS, and MDRS-2 scores prior to first drug administration will be used as the baseline value in the model and to calculate change from baseline clinical scores. To calculate the change from baseline at week 54, the clinical scores at week 54 will be used. Missing values will be imputed using the last observation carried forward (LOCF) method, including clinical scores on the discontinuation visit. Baseline values will not be carried forward. This methodology will be applied to all treatment groups (liraglutide and placebo).

The assumptions of normality and homogeneity of variance will be explored using probability and residual plots. Data will be tested for normality using the Shapiro-Wilk test. If any of the assumptions are found to be violated, an appropriate transformation of the data or non-parametric test will be considered.

Sensitivity analyses of the primary efficacy endpoint will be performed on the PP population using the same methods described for the FAS population. Summaries of clinical scores, including the change from baseline, will be presented at each visit (i.e., baseline, Week 28, and Week 54). No adjustments have been made to the Type I error rate for the multiple comparisons of the primary or secondary endpoints since this is a phase II study; therefore, results should be interpreted appropriately. No statistical testing within treatment groups will be performed.

Inferential Statistics on Secondary Efficacy Parameter(s): Analyses of secondary efficacy endpoints will be conducted on the FAS and PP populations. The following secondary efficacy endpoints will be analyzed using an approach similar to that described for the primary efficacy endpoints:

- Change in PDQ-39 scores for each domain and total score between baseline and week 54.
- Change in Delis-Kaplan Executive Function System (DKEFS) between baseline and week

54
- Change in Geriatric Depression Scale between baseline and week 54
- Change in Parkinson's Anxiety Scale scores between baseline and week 54

The safety outcomes of this study will be the following:
- Adverse events
- Serious adverse events
- Clinical laboratory parameters
- Geriatric Depression Scale
- Vital signs
- Physical examination.
- 12-lead ECGs

Interim Analysis: No interim efficacy analyses are planned for this study. However, after intervals during the study (for example, when approximately 25% of the subjects (ca. 15 subjects) have been randomly assigned to the treatment groups and again when 25%, 50%, 75%, and 100% of the planned number of subjects have reached 12 weeks of dosing (week 14 visit)) the DSMB will conduct a review of safety data and will provide the Investigator with recommendations regarding the conduct of the study. In particular, the DSMB will review safety data (including thyroid and liver function, serum amylase, and fasting serum glucose and insulin) when the first 25% of subjects have been enrolled and will make recommendations to the Investigator as to whether or not there are any concerns which warrant discontinuation of enrollment in any given treatment arm and/or the study overall.

The DSMB will be composed of four members, selected among clinicians and biostatisticians at Cedars Sinai Medical Center with expertise in Parkinson's disease, glucose metabolism, pancreatitis and current clinical trials conduct and methodology. No member of the DSMB will have direct involvement in the conduct of the study. Furthermore, no member will have financial, proprietary, professional, or other interests that may affect impartial, independent decision-making by the DSMB. The research coordinator or other PI designee will prepare blinded data including interim and cumulative data regarding study-related adverse events; quality, completeness, and timeliness of data collection; adequacy of compliance with goals for recruitment and retention; adherence to the protocol and protocol violations. The DSMB will conclude each review with their recommendations to the PI as to whether the study should continue without change, be modified, or terminated. Recommendations regarding modification of the design and conduct of the study could include: 1) modifications of the study protocol based upon the review of the safety data; 2) suspension or early termination of the study because of serious concerns about subjects' safety, inadequate performance or rate of enrollment.

DATA/SAMPLE HANDLING AND RECORD KEEPING: The Investigator will delegate responsibility for data entry and quality to a named individual who will be part of the trial team, in accordance with the Data Protection Act of 1998. The Investigator will be responsible for data analysis done independently of data entry. The Cedars-Sinai Core Statistical team will be on hand with additional advice.

The plasma samples for EV analysis will be stored in the Advanced Health Sciences Pavillion building in a -80^{o}C freezer.

ETHICS: The trial will comply with ICH GCP and applicable regulatory requirements and proceed in

accordance with the Declaration of Helsinki. Prior to the start of the study, the Investigator will apply for approval of the study from the Cedars-Sinai IRB. All documents required by the IRB and the regulatory authority will be submitted. Any notification/submission must be dated and contain sufficient information to identify the relevant protocol. The study will only commence after receipt of written approval from the Cedars-Sinai IRB. The Investigator designee is responsible for maintaining the approval documents in the Investigator study files. The Investigator will report promptly to the IRB any new information that may adversely affect the safety of the patients or the conduct of the trial. At the end of the study, the Investigator is obligated to inform the IRB in writing that the study has ended and no further activities regarding this protocol will be conducted at the site

STUDY SCHEDULE: The trial will be conducted in the Neurology Department of the Cedars-Sinai Medical Center in Los Angeles, CA, according to the following schedule of events. Enrollment is expected to start (FPFV) on July 1st, 2016 and conclude (LPLV) on June 30th 2018.

Screening and information visit
The informed consent will take place after the study candidate has received extensive oral and written information about the study, including the risks associated with the study procedures and study-drug, prior to any study-related activity, including screening procedures. After consenting, detailed information will be collected on all study candidates, including demographics and relevant medical history, MDRS-2 screening, BDI-II screening, physical and neurological exams, routine vitals (height, weight, blood pressure in clino- and orthostatic positions), an EKG, and blood and urine tests. For women of child-bearing age, a serum pregnancy test will also performed.

Baseline evaluation visit
After confirmation of eligibility criteria, subjects will subsequently be randomized to one of the two treatment arms. They will then undergo MDS-UPDRS OFF and ON testing and Neuropsychological testing (ON). The practically defined OFF condition will be considered an overnight withdrawal of dopaminergic medications for 12 hours while ON evaluation will be performed 1 hour ($\pm$ 15 minutes) after the administration of the usual morning dose of dopaminergic medications. The first daily dose of 0.6 mg and/or placebo, including injection instructions, will be administered at the end of the baseline visit. The baseline visit is to be conducted within 30 days of screening.

Commence treatment: 2-week titration
The following two visits will be dedicated to medication titration, up to 1.2mg (Week 1) and 1.8 mg (Week 2), as tolerated. At every visit, study drug compliance will be reviewed. Adverse events will be assessed and concomitant medications, routine vitals, and ECG, and blood and urine tests will be collected. A phone interview will be conducted following titration to determine which dosage (1.2 mg or 1.8 mg) the patient will remain on for the duration of the study.

Follow-up visit 1 and 2
4 and 12 weeks after titration is completed, a follow-up visit will establish whether the subjects are tolerating study medications, including an assessment of adverse events, concomitant medications, routine vitals, an ECG, and blood and urine tests.

Follow-up visit 3 (6 month)
26 weeks after completing titration, subjects will undergo repeat MDS-UPDRS OFF and ON testing and

Neuropsychological testing (ON) in addition to an assessment of adverse events, concomitant medications, routine vitals, an ECG, and blood and urine tests.

Follow-up visit 4 (9 months)
36 weeks after titration is completed, a follow-up visit will establish whether the subjects are tolerating study medications, including an assessment of adverse events, concomitant medications, routine vitals, and ECG, and blood and urine tests.

End visit
52 weeks after completing titration, subjects in both treatment arms will undergo an exit visit including MDS-UPDRS OFF and ON testing and neuropsychological testing (ON) in addition to an assessment of adverse events, concomitant medications, routine vitals, an ECG, and blood and urine tests. At this stage, study treatment will be discontinued.

Post-study visit
4 weeks after the end of the study, a phone interview will be completed to assess adverse events which were ongoing at the end visit (Week 54) and concomitant medications.

Post-study follow-up (6 month)
26 weeks after completing the active phase of the study (end visit), subjects originally assigned to both treatment arms will undergo a follow-up visit including MDS-UPDRS OFF and ON testing and neuropsychological testing (ON) in addition to an assessment of adverse events which were ongoing at the end visit (Week 54), concomitant medications, routine vitals, an ECG, and blood and urine tests. At this stage, enrolled subjects will no longer be on study treatment. However, the study team may continue to follow subjects who opt to continue liraglutide off-label.

Post-study follow up (12 month)
52 weeks after completing the active phase of the study (end visit), subjects originally assigned to both treatment arms will undergo a follow-up visit including MDS-UPDRS OFF and ON testing and neuropsychological testing (ON) in addition to an assessment of adverse events which were ongoing at the end visit (Week 54), concomitant medications, routine vitals, an ECG, and blood and urine tests. At this stage, enrolled subjects will no longer be on study treatment. However, the study team may continue to follow subjects who opt to continue liraglutide off-label.

STUDY DRUGS AND MATERIALS:

Study Medications: Liraglutide 6 mg/ml (Novo Nordisk A/S) will be self-administered subcutaneously once daily at a maximum dose of 1.8 mg after a 2 week titration schedule. Placebo will be administered according to the same schedule (Table 2). At baseline, subjects will be dispensed drug pens preset to deliver 0.6 mg of either liraglutide or vehicle per daily dose. At week 1 visit, after being assessed for tolerability by the investigator, subjects will be titrated to 1.2 mg of either liraglutide or vehicle per daily dose. The same procedure will be repeated at week 2, when subjects will be titrated to 1.8 mg of either liraglutide or vehicle per daily dose, as tolerated. A subject that cannot tolerate at least a 1.2 mg dose will be excluded from the study. Subjects that cannot tolerate the 1.8 mg dose, will be offered to continue at the 1.2 mg dose. The project will include a washout period at study end.

Table 2

Titration Schedule		
Week	Drug Group	Placebo Group
	Liraglutide Dose	Vehicle Dose
Baseline	0.6 mg	0.6 mg
Week 1	1.2 mg	1.2 mg
Week 2 - 54	1.2 or 1.8 mg	1.2 or 1.8 mg

Packaging and Labeling of Study Medications: Packaging and labeling of the trial products will be handled by the manufacturer (Novo Nordisk A/S). Liraglutide and its vehicle will be administered via pens supplied by the manufacturer (Novo Nordisk A/S).

Storage and Drug Accountability of Study Medications: The research pharmacist will inventory and acknowledge receipt of all shipments of the study drug. The study drug will be kept in a locked area with restricted access. The study drug will be stored in a refrigerator between 36ºF to 46ºF (2ºC to 8ºC), in a secure location. After initial use, the pen can be stored for 30 days at controlled room temperature (59°F to 86°F; 15°C to 30°C) or in a refrigerator (36°F to 46°F; 2°C to 8°C), with the pen cap on when not in use. The pen should be always protected from excessive heat and sunlight.

The PI, pharmacist, or designee is responsible for maintaining accurate records of the receipt, dispensing, proper storage, and return of all investigational materials. Cedars Sinai Investigational Drug Pharmacy will be the designee to control on-site storage conditions of the drug prior to dispense. Temperature logs are maintained and archived indefinitely. At study closure, printed logs are provided in the pharmacy binder for the duration of study drug storage. The PI, pharmacist, or designee may dispense study drug only to subjects enrolled in the study. Under no circumstance will the PI, pharmacist, or designee allow study drug to be used other than as directed by the protocol. When a shipment is received, the research Pharmacist or designee must verify the quantities received and return the acknowledgement to the Drug Manufacturer or designee. The study drug accountability record includes the identification of the subject to whom the drug is dispensed, the lot number on the pen, the date of dispensing, and any returned or unused drug. An account must be given of any discrepancies. These records must be readily available for inspection by any relevant Health Authority at any time. The Investigational Drug Pharmacy will destroy unused drug following institutional drug destruction policy and will maintain a record of destruction of the drug incinerated off-site via the hospital's contracted pharmaceutical waste vendor, Stericycle.

Randomization and Blinding: The study drug will be administered according to the clinical study

protocol only to subjects who have signed the informed consent form (ICF).

At the end of the Baseline visit (see Study schedule), each subject meeting entry criteria will be randomly assigned to one of the two treatment groups in a 2:1 ratio (liraglutide or placebo).
A pre-determined randomization list generated by our biostatistics team will be given to and maintained by pharmacy staff. Once assigned, codes will not be reused for any other subjects in the study. In data collection records, subjects will be designated only by these de-identified codes. The randomization schedule so generated will be maintained by the investigational pharmacist, who will remain the only unblinded study staff member.

The liraglutide and matching placebo injection pens will be identical and indistinguishable to the Investigators, the subjects or others assisting in treatment administration. In accordance with the double-blind design, all these individuals will remain blinded to study treatment and will not have access to the randomization (treatment) codes except under the circumstances described below.

Breaking of Blinded Codes: The blinding code may be broken only in exceptional circumstances when knowledge of the study drug is essential for treating the subject, such as in case of an adverse event. Code breaking can be performed by the PI or authorized designee at any time by opening a sealed envelope containing unblinding information provided by the pharmacy staff. The Investigator or the designee who breaks the blind must record the date and the reasons for doing so in the source documentation and in the subject's medical records

CONCOMITANT ILLNESSES AND MEDICATIONS: See Inclusion and Exclusion Criteria

ADVERSE EVENTS:

Collection, Recording, and Reporting of Adverse Events (AEs): The collection of non-serious AE data and serious adverse event (SAE) information commences following the subject's written consent to participate in the study. An AE is any untoward medical occurrence in a clinical investigation subject administered an investigational product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product (study drug). An Adverse Reaction (AR) is an AE for which a causal relationship to the trial product is at least possible, i.e. a causal relationship is conceivable and cannot be dismissed.

The description of each AE will identify the subject, date of onset, the date of resolution, the severity of the event, the action taken regarding study drug, the outcome of the event, and the relationship of the event to study drug. AE information will be entered in the CRF within 7 days of the information becoming available in order to ensure timely reporting to the Sponsor.

Severity of reported AEs will be evaluated using the following guidelines:
- Mild: The subject is aware of the AE but it is easily tolerated.
- Moderate: The AE causes the subject discomfort and interrupts the subject's activities of daily life.

- Severe: The AE is debilitating or results in the inability of the subject to perform the activities of daily life.

The Investigator will use the following definitions to evaluate the relationship of each AE to the study drug:

Definitely: An AE that follows a reasonable temporal sequence from administration of the study drug (including the course after withdrawal of the study drug) and that satisfies any of the following:
• reappearance of a similar reaction upon re-administration (positive rechallenge);
• toxic level of the study drug as evidenced by measurement of study drug concentrations in blood or other bodily fluid.

Probably Related: An AE that follows a reasonable temporal sequence from administration of the study drug (including the course after withdrawal of the study drug) and for which involvement of factors other than the study drugs, such as underlying diseases, complications, concomitant drugs, and concurrent treatments, can reasonably be excluded. This may also include positive dechallenge, in which the event abates upon discontinuation of the study drug.

Possibly Related: An AE that follows a reasonable temporal sequence from administration of the study drug (including the course after withdrawal of the study drug) and for which possible involvement of the study drug can be suggested (for example, previous similar reports or pharmacologic actions of the study drug) although factors other than the study drug, such as underlying diseases, complications, concomitant drugs, and concurrent treatments may also be responsible.

Unlikely: An AE that, while not necessarily unrelated to the study drug, does not appear to follow a reasonable temporal sequence from administration of the study drug or that appears to be reasonably explained by other factors, such as underlying diseases, complications, concomitant drugs, and concurrent treatments.

Not related: An AE that does not follow a reasonable temporal sequence from administration of the drug or that can reasonably be explained by other factors, such as underlying diseases, complications, concomitant drugs, and concurrent treatments.

The liraglutide US Prescribing Information document will be used for evaluations of expectedness, as agreed with the drug manufacturer.

An **SAE** is an experience that, at any dose, results in any of the following:
• Death;
• A life-threatening event in which the patient is at risk of death at the time of the event. Note: it does not refer to an event that hypothetically might have caused death if it was more severe.
• In-patient hospitalization or prolongation of an existing hospitalization;
• Persistent or significant disability/incapacity;
• Congenital anomaly or birth defect in the offspring of the subject (whether the subject is male or female);
• Need of medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure;

• An important medical event: Those events that may not be immediately life-threatening or result in death or hospitalization but based upon appropriate medical and scientific judgment may jeopardize the subject or may require intervention to prevent one of the serious outcomes listed above.
• Suspicion of transmission of infectious agents must always be considered an SAE.

A Serious adverse reaction (SAR) is an adverse event that fulfils both the criteria for a Serious Adverse Event and the criteria for an Adverse Reaction.

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is a SAE that is unexpected and regarded as possibly or probably related to the trial/study product by the investigator.

SAEs require expeditious handling to comply with regulatory requirements. Any SAE, SAR or SUSAR occurring in a clinical study subject will be reported to the Institutional Review Board (IRB) within 10 days of the Investigator having knowledge of the SAE. In addition, the investigator will copy Novo Nordisk A/S (NN) when expediting SARs or SUSARs to health authorities and will report all SARs related to NN Product to the local NN affiliate safety department. The submission to NN will be within day 15 from the investigator's first knowledge about a valid case. The Investigator or other qualified individual at the investigative site will complete an SAE form including study name, name of the reporter, subject identification (e.g. subject number, initials, gender, age), event (preferably diagnosis), study drug, most recent date of administration, the Investigator's causality assessment to study drug (as outlined above) and outcome.

Follow-up of Adverse Events: If a subject experiences an SAE or AE, the subject will receive appropriate treatment and supportive care, as necessary, and the PI will continue to follow-up until there is a return to the subject's baseline condition, or until a clinically satisfactory resolution is achieved.

Pregnancy Beginning During Course of Trial: Female study subjects will be advised by the Investigator to inform him immediately if she suspects she may be pregnant up to 30 days after the last dose of study drug.

Male study subjects will be advised by the Investigator to inform him immediately if he suspects his partner became pregnant up to 30 days after the last dose of study drug.

When a study subject reports a possible pregnancy to the PI after the start of drug administration, study drug should be stopped immediately and a pregnancy test should be arranged for the subject (or partner) by the PI within 7 days of the pregnancy being reported. In the case of pregnancy, the PI must immediately notify the Drug manufacturer of this event and report the pregnancy in the source documentation. This includes a study subject as well as the partner of a study subject who becomes pregnant while the subject was receiving study drug. Every attempt will be made to follow the pregnancy to conclusion to obtain information regarding the outcome. All pregnancies in trial subjects exposed to the study drug will be reported to the drug manufacturer (Novo Nordisk A/S).

Precautions/Over-dosage: Based on previous studies with liraglutide in patients with type-2 diabetes,[76] the following percentages of cases are expected to experience mild-to-moderate nausea (5-40%), diarrhea (17%), constipation (10%), and/or headache (9%). Subjects will be asked to report these or any other adverse effects when they arise and during each of the scheduled visits (see Study Schedule).

Overdoses have been reported in clinical trials and post-marketing use of liraglutide, with resulting effects including severe nausea and vomiting. In the event of over-dosage, appropriate supportive treatment will be initiated according to the subject's clinical signs and symptoms.

LIABILITY AND SUBJECT INSURANCE: A research-related injury or illness is a direct result of the study drug or a procedure performed only as a part of the study and is not part of standard clinical medical treatment. Cedars-Sinai has no plans to pay for costs associated with the treatment of research-related injury or illness. If a study subject will need treatment for a research-related injury or illness, Cedars-Sinai Health System will make every effort to seek reimbursement from his/her health plan. The subject will be responsible for any deductibles and co-payments required under his/her health plan and for any claims ultimately denied by the health plan. Financial assistance may be available under Cedars-Sinai's Charity Care Policy and Procedure. Losses such as lost wages will not be paid.

PREMATURE TERMINATION OF STUDY: In case the study is terminated early due to the discovery of an unexpected, significant, or unacceptable risk/safety concern, regardless of how far along the subject has reached in their study follow-up, subjects will be asked to return all unutilized pens to the PI. The study site will then return investigational drug to the drug manufacturer (See also Study Drug Accountability).

PUBLICATION PLANS: The intention of the study will be to publish the results of the complete study at conclusion. All information obtained during the conduct of this study will be regarded as confidential but no written permission from the study funding sources is required prior to disclosing any information relative to this study. A formal publication of data collected as a result of the study will be considered a joint publication by all investigators and appropriate personnel. Authorship will be determined by mutual agreement. Submission to the funding source(s) for review and comment will be intended solely to ensure concurrence regarding data, evaluations, and conclusions, and to provide an opportunity to share with them any new or unpublished information of which they may be unaware.

Schedule of events

Procedures	Screening	Baseline	Week 1	Week 2	Week 3	Week 6	Week 14	Week 28	Week 40	Week 54	Week 58	Week 80	Week 108
Day	Within 30 days of baseline	1	8 + 3	(7+3 days from Week 1)	(7+3 days from Week 2)	42 ± 7	98 ± 7	196 ± 7	280 ± 7	378 ± 7	406 ± 7	560 ± 7	756 ± 7
Sign Consent	x												
Demographics	x												
Medical History	x												
Pregnancy Test	x												
Physical and Neuro Exam	x												
EKG	x		x	x		x	x	x	x	x		x	x
Vitals	x	x	x	x		x	x	x	x	x		x	x
Blood and Urine samples[1]	x		x	x		x	x	x	x	x		x	x
Blood for Biomarker Analysis	x							x		x		x	x
Review of eligibility		x											
MDS-UPDRS OFF[2]		x						x		x		x	x
MDS-UPDRS ON		x						x		x		x	x
BDI-II	x												
NMSS		x						x		x		x	x
MDRS-2	x							x		x		x	x
Neuropsych tests		x						x		x		x	x
Treatment Randomization and First Dose		X (0.6) Placebo	X (1.2) Placebo	X (1.8) Placebo									
Drug Administration Training		x	x	x									
Dispense Study Drug		x	x	x		x	x	x	x				

Review Study Drug Compliance			**x**	**x**		**x**	**x**	**x**	**x**	**x**			
AE Assessment	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**
Assessment of Concomitant Medications		**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**
Final dosage determination[3]					**x**								

1)Lab tests – schedule across study:

- Screening: HbA1C, lipid panel, fasting insulin, CBC, CMP, amylase, lipase, calcitonin, urinalysis, blood for biomarker analysis
- Weeks 1, 2, 6, 14, 40: CBC, CMP, amylase, lipase, calcitonin, urinalysis
- Weeks 28, 54, 80, 108: HbA1C, fasting insulin, CBC, CMP, amylase, lipase, calcitonin, urinalysis, blood for biomarker analysis
- Note: Lab tests may be repeated if the study team has concerns regarding test result accuracy or subject safety

2)OFF Medication assessments – withhold all PD meds and Liraglutide for at least 12 hours (overnight) before clinic visit.

3) Dosage may be changed after Week 3 per PI discretion

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