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# Draft qualification opinion on molecular neuroimaging of the dopamine transporter as biomarker to identify patients with early manifest Parkinsonism in Parkinson's disease

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Comments should be provided using this template. The completed comments form should be sent to qualification@ema.europa.eu


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| **Keywords** | Biomarker, molecular neuroimaging, Parkinson's disease |


[1] Last day of relevant Committee meeting.
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30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
**Telephone** +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
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## Introduction

Qualification of Molecular neuroimaging of the dopamine transporter as biomarker to identify patients with early manifest Parkinsonism in Parkinson's disease

On 05 April 2017 the Applicant Critical Path Global Ltd. requested qualification opinion for Molecular neuroimaging of the dopamine transporter as biomarkers to identify patients with early manifest Parkinsonism in Parkinson's disease.

The procedure started during the SAWP meeting held on 02 - 05 May 2017.

At its meeting on 06 – 09 June 2017, the SAWP adopted a list of issues to be addressed by the Applicant during the discussion meeting. The discussion meeting with the Applicant took place on 04 July 2017.

During its meeting held on 29 August – 01 September 2017, the SAWP agreed on the draft opinion to be given to the Applicant. During its meeting held on 11 – 14 September 2017, the CHMP adopted the draft opinion to be given to the Applicant. The draft Opinion was published for consultation.

During its meeting held on 23 – 26 October 2017, the SAWP agreed on the opinion to be given to the Applicant. During its meeting on 06 – 09 November 2017, the CHMP adopted the final Opinion to be given to the Applicant.

## Executive summary

Critical Path Global Ltd.'s Critical Path for Parkinson's (CPP) is a multinational consortium of the Critical Path Institute supported by Parkinson's UK and industry. This broad collaboration of pharmaceutical companies, government agencies, academic institutions, and charities aims to accelerate the development of therapies for Parkinson's disease (PD). The CPP Imaging Biomarker team aims to achieve a qualification opinion by EMA Committee for Medical Products for Human Use (CHMP) for the use of low baseline Dopamine Transporter levels for subject enrichment in clinical trials in early stages of PD.

This package reports the results of the Critical Path Global Ltd. CPP Imaging Biomarker team's analysis of baseline levels of Dopamine Transporter (DAT) density as assessed by Single-Photon Emission Computed Tomography (SPECT) neuroimaging as an enrichment biomarker in clinical trials for the treatment of PD. The biomarker proposed for qualification is molecular imaging of DAT, a transporter protein that is located on the presynaptic nerve terminal of dopaminergic neurons. Molecular imaging of the DAT protein represents a viable method of assessing the integrity of dopamine nerve terminal function in living human brain.

The aim of this work is to demonstrate the predictive accuracy of visual assessment of DAT neuroimaging scans at baseline for identifying those subjects with high likelihood of progressing in clinical motor disability. By excluding subjects from clinical trials who are classified as having a "Scan Without Evidence of Dopaminergic Deficit" (SWEDD), subjects more likely to have PD can be more accurately identified for inclusion in future clinical trials. Patients with striatal dopamine deficit will be identified at the earliest signs of clinical motor impairment, when candidate therapeutic drugs presumably would more effectively disrupt the neurodegenerative process and declining clinical trajectory. It is proposed that confirming reduction of DAT expression levels by SPECT neuroimaging of subjects with early motor deficits is a useful means of enriching clinical trials of PD therapeutic agents, as this facilitates excluding patients who are unlikely to show disease progression from enrolment in a PD clinical trial.

Patient-level imaging and clinical data were acquired and analyzed from two large multicenter global PD clinical cohorts focused on patients at early motor stages. The studies include a large randomized, double-blind, placebo-controlled, clinical trial (Parkinson Research Examination of CEP-1347 Trial - PRECEPT) and a longitudinal observational cohort focused on biomarker discovery and validation (Parkinson's Progression Markers Initiative - PPMI).

In the integrated PPMI and PRECEPT studies, DAT levels assessed visually at baseline accurately predicted that SWEDD subjects were unlikely to progress in motor disability. Results suggested that SWEDD subjects have a statistically significant slower rate of motor worsening compared to subjects with DAT deficit as shown by the harmonized Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) score.

The imaging biomarker is appropriate for assessing dopamine deficiency consistent with Parkinsonism as a tool to aid in subject selection for clinical trials. Reproducibility and reliability have been addressed by including a detailed methodology section recommended for use by sponsors. These data can be used to deploy the biomarker confidently in clinical trials of PD subjects soon after clinical selection to enable early intervention, sparing subjects without dopaminergic deficit from being exposed to test therapeutic candidates. This document communicates the degree of enrichment one expects from using the biomarker in prospective clinical trials according to the proposed context-of-use.

## Background information as submitted by the applicant

### Background on the disease

Drug development for the treatment of PD is being pursued aggressively by industry, biotech and non-profit organizations. Challenges that all drug developers face for PD therapeutics include the prolonged duration of disease progression, the heterogeneity of the patient population, the risk of adverse drug reactions in an elderly patient population and paucity of biomarkers to differentiate subtypes of Parkinsonism. There is increasing recognition that novel disease-modifying therapies will be most efficacious if treatment is initiated very early in the course of the disease. Significant challenges exist in advancing treatments for very early-stage PD subjects in that it is difficult to accurately diagnose patients based upon clinical evaluations alone. Clinical symptoms of early motor PD overlap with many different conditions and the true percent of atypical Parkinsonism or other non-PD cases in legacy PD clinical trials is still not known. Novel biomarker approaches are needed to accurately identify PD patients for subject selection in clinical trials.

### Background on the biomarker

The biomarker proposed for qualification is molecular imaging of DAT, a transporter protein that is located on the presynaptic nerve terminal of dopaminergic neurons. Reductions of DAT radiotracer binding correlate with the loss of presynaptic nigrostriatal neurons. Ligands specific for in vivo imaging of DAT directly measure the functional integrity of the dopamine nerve terminal and are used to monitor neurodegeneration in both nonclinical and clinical studies. Significant clinicopathologic findings illustrate that reductions in DAT assessed by neuroimaging reflect dopaminergic nerve terminal degeneration in animal models and in patients with Parkinson's disease and that such reductions precede the onset of clinical symptoms.

At present, the ligand approved by regulatory agencies for use in humans is the DAT-selective radioligand [$^{123}$I]N-omega-fluoropropyl-2β-carbomethoxy-3β-[4-iodophenyl] nortropane (FP-CIT) [the brand name for this biomarker in the European Union (EU) is DaTSCAN$^{TM}$]. $^{123}$I-ioflupane ($^{123}$I-FP-CIT) is a SPECT tracer, licensed by the European Medicines Agency and available in Europe since 2000. In the United States, $^{123}$I-ioflupane was approved by the Food and Drug Administration in January 2011

and is commercially available (1). The approved use for $^{123}$I-ioflupane is to aid in the differential diagnosis of Parkinson's disease in clinical practice.

This document is aimed at qualification of the biomarker itself, as opposed to a specific tracer. Multiple tracers exist for in vivo imaging of dopamine transporter levels yet only $^{123}$I-ioflupane [($^{123}$I-FP-CIT); DaTscan™] is approved for use in EU and US. Other tracers are used as research-only tools. Therefore, the focus for this technical document is on the approved tracer, given that it will be the tool of choice for prospective clinical trials and has been employed successfully in the multisite PPMI observational cohort study.

**Role in Drug Development**

There is an urgent need for biomarkers to be used as tools that can be successfully employed in trials to enable patient selection, proof of mechanism and to monitor effects of new drug candidates on disease progression. While the field of PD has lagged behind that of other disease areas in terms of biomarker discovery and validation, rich data-driven approaches focused on biomarker identification have been well underway for several years [e.g., (2) (3)] and many clinical trials employing candidate biomarkers exist.

Multiple neuroimaging ligands exist for markers of the dopamine neurotransmitter system (4) and represent in vivo tools to aid in clinical trials for PD. Correlations between markers of dopaminergic function as assessed by PET have been reported in the same PD patients (5) suggesting imaging radiotracers of presynaptic dopamine nerve terminals reflect similar functional deficits.

Neuroimaging assessment of Dopamine Transporter levels has been widely used and serves as a reliable index of the integrity of dopamine nerve terminal function in living human brain. Reductions of DAT levels as assessed by SPECT neuroimaging is intended to be used as an adjunct to clinical assessments for the purposes of enriching clinical trials with subjects that are more likely to demonstrate disease progression. It is proposed that the use of the DAT neuroimaging biomarker will facilitate enrollment of a more homogenous cohort of patients with PD and increase the probability of success of a trial.

**Proposed context-of-use (cou) statement**

**General Area:**

- Enrichment biomarker for clinical trials in early motor Parkinson's disease.

**Target Population for Use:**

Patients with early motor PD, defined by the UK Brain Bank Criteria (6) as outlined below:

- Having at least two of the following: resting tremor, bradykinesia, rigidity (must have either resting tremor or bradykinesia); OR either asymmetric resting tremor or asymmetric bradykinesia.
    - Based on the above criteria, combinations could include: resting tremor/bradykinesia, bradykinesia/rigidity, and resting tremor/rigidity.
    - Symptom(s) or signs may include bradykinesia, a 4-6 Hz resting tremor, muscle rigidity, or postural instability not caused by primary visual, vestibular, cerebellar or proprioceptive dysfunction.
- Hoehn and Yahr Stage I or II at baseline.

- Although postural instability is a common feature in PD, based on the inclusion criterion of Hoehn and Yahr Stage I or II, postural instability would not be expected in the target population.

**Stage of Drug Development for Use:**

- All clinical stages of early PD drug development, including proof of concept, dose-ranging through to confirmatory clinical trials. This is not intended for candidate therapies for more advanced stages of PD such as drugs to treat L-Dopa induced dyskinesias.

**Intended Application:**

Purpose: The objective of this project is to apply DAT imaging as a biomarker tool to enrich subjects for clinical trials in early symptomatic PD by identifying subjects with a DAT deficit for possible inclusion into the study and excluding subjects who are unlikely to progress due to the lack of dopamine deficiency in the brain. The DAT imaging is intended to be used after the clinical criteria for early PD have been satisfied. Points 1-4 describe the process and points 5-9 describe how the information obtained from steps 1-4 will be applied.

1. Potential candidates for PD clinical trials will be evaluated for the presence of at least two motor signs of PD as defined in the target population descriptions in the section Aligning Target Patient Populations (Appendix) (according to the PPMI and PRECEPT criteria).

2. Those individuals will then be evaluated according to the UK Brain Bank (6) step 1 Criteria for PD.

3. If the two conditions above are met, subjects will undergo the trial-specific inclusion/exclusion criteria; and further clinical assessment for atypical Parkinsonian syndromes.

4. As a final step in the subject-selection process, molecular imaging of DAT will be performed to detect the presence or absence of DAT-deficiency and identify and exclude subjects defined as SWEDDs.

5. Such baseline categorization of DAT-deficiency can be applied as an enrichment biomarker that, in combination with specific clinical signs, can more accurately predict disease progression of motor disability in early PD patients. Such progression will be expressed by the motor scores of the Unified Parkinson's Disease Rating Scale (UPDRS) or Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) scales, which constitute reliable outcomes of disease progression in PD.

6. Baseline categorization of DAT-deficiency can be applied as a subject selection biomarker to enrich trial populations with patients more likely to progress in the motor scores of UPDRS or MDS-UPDRS scale (parts II and III) over the course of clinical trials, which may be up to two years in duration. The purpose is to exclude patients that are unlikely to show disease progression (SWEDD), and consequently to increase the probability of the trial conclusively demonstrating the efficacy of a drug in clinical trials for therapeutic interventions for early PD. Those individuals who are not SWEDDs and who meet all the other selection criteria will be enrolled into the trial and randomized as per the specified study design.

7. The use of DAT imaging would allow the exclusion of subjects unlikely to have the diagnosis of PD and therefore prevent them from unjustified exposure to experimental PD-specific therapies with inherent safety and tolerability risks without anticipated benefit.

8. The application is relevant to both symptomatic and disease modifying candidate therapies for early PD and is independent of the mechanism of action of the new drug.

9. The use of DAT imaging for diagnostic applications is out-of-scope for this proposed COU.

**Critical Parameters for the Context-of-Use:**

- The context-of-use specifies that reductions of DAT as assessed by SPECT neuroimaging will be utilized as an adjunct to clinical assessments for the purposes of enriching the patient population with subjects who have increased likelihood of having idiopathic PD. The subjects will have an objectively confirmed motor impairment with alternative identifiable causes of motor impairment appropriately excluded through clinical means prior to the use of DAT neuroimaging.

- SPECT neuroimaging procedures and methodologic aspects of imaging will be performed qualitatively in accord with the tracer manufacturer's specifications and consistent with the methods currently employed in the multi-site PPMI study. The proposed analysis of DAT SPECT images is by visual assessment by trained, blinded readers and analysis is to be carried out by a single site. Such processes are expected to generate sufficiently accurate, reproducible and robust assessment of DAT neuroimaging to facilitate clinical trial enrichment.

Figure A1 (Appendix) illustrates a proposed flow diagram for use of DAT imaging in PD clinical trials.

**Imaging methodology**

CPP core imaging team experts have collaborated to develop technical recommendations aimed at catalyzing reliable and reproducible use of the imaging biomarker by sponsors employing DAT imaging at baseline for subject selection according to the context-of-use. Note that the qualification opinion is aimed at qualification of the biomarker itself as opposed to a specific tracer. Multiple tracers exist for in vivo imaging of dopamine transporter levels yet only 123I-ioflupane [(123I-FP-CIT); DaTscanTM] is approved for use in EU and US. Other tracers are used as research only tools. Therefore, the focus is on the approved tracer given that it will be the tool of choice for prospective clinical trials and has been employed successfully in the multisite PPMI observational cohort study.

SPECT neuroimaging procedures and methodologic aspects of imaging will be performed qualitatively in accord with the tracer manufacturer's specifications and consistent with the methods currently being employed in the multi-site PPMI study. Such processes are expected to generate sufficiently accurate, reproducible and robust assessment of DAT neuroimaging to facilitate clinical trial enrichment.

This guideline covers the indications, technical aspects, interpretation, and reporting of DAT SPECT scans with $^{123}$I-ioflupane. The summary consists of information originating from the Society of Nuclear Medicine, previously accepted and published in peer reviewed literature (8) and also integrates the recommendations of the European Association of Nuclear Medicine (9). Additional sources for information include the PPMI imaging technical manual (www.ppmi-info.org/wp-content/uploads/2010/07/Imaging-Manual.pdf) and imaging technical recommendations from the manufacturer, GE Healthcare (GE website; http://us.datscan.com/wp-content/uploads/2016/07/JB39854US-US-DaTscan-Protocol-Manual-digital-secure.pdf).

A more detailed description of the imaging methodology for reliable use of DAT imaging as an enrichment biomarker in PD clinical trials is outlined in the Imaging Methodology section of the appendix.

**Data analysis methods**

**Data Sources**

Two PD clinical studies were used for analyses.

PRECEPT was a Phase 2/Phase 3, multicenter, randomized, double-blind, placebo-controlled, dose-finding study. This study aimed at neuroprotection sought to determine if treatment with the candidate Mixed Lineage Kinase inhibitor, CEP-1347 delayed the time-to-onset of disability sufficient to require dopaminergic therapy in patients with early Parkinson's disease who did not receive or require dopaminergic therapy for symptomatic control of their disease at study start.

The PPMI is an ongoing multicenter observational trial supported by a consortium of academic centers, Parkinson's disease foundations, and pharmaceutical and biotechnology companies to collectively design and fund the identification and validation of Parkinson's disease progression markers (2).

PRECEPT and PPMI represent uniquely rich cohorts of well characterized subjects with early stage (de novo) PD where subject-level data is available to CPP for analyses to support regulatory science goals. Both studies include similar patient populations from multicenter global sites with application of DAT imaging at baseline and long term clinical follow up. The use of both observational and randomized clinical trial (RCT) populations aides in the confidence of predictability of the results to prospective trial populations that align with the proposed context-of-use. Comprehensive descriptions of the PRECEPT and PPMI PD clinical studies are found in the Data Sources section of the appendix. Within the scope of this analysis were (a) the PD cohort in PPMI; (b) the SWEDD cohort in PPMI; and (c) the placebo arm in PRECEPT.

Patient-level data from the PRECEPT (10) clinical trial and the PPMI (11) clinical study were transformed to CDISC standard format using SAS software (SAS Institute, Cary, NC, USA) and used to populate the database. Rigorous quality control steps were taken at the completion of data mapping and transformation to ensure accuracy, consistency and conformance to the standards of the resulting datasets.

Subjects were to be diagnosed with early stage PD defined as (a) being in a Hoehn and Yahr stage I or II at baseline, and (b) having at least two of the following signs: resting tremor, bradykinesia, rigidity; or either asymmetric resting tremor or asymmetric bradykinesia. Meeting such criteria for early stage PD was part of the inclusion criteria for the aforementioned cohorts or arm in PPMI and PRECEPT. Criteria for data exclusion were: (a) Observations with a missing value for the dependent variable; (b) Observations that occurred in time before baseline assessments (e.g., screening); (c) Observations that occurred in time equal to or greater than 25 months; (d) Subjects with missing DAT biomarker status according to visual interpretation.

**Time Metric and Dependent Variable**

The time metric was the time in the study in months. The dependent variable was the harmonized UPDRS and MDS-UPDRS Part III score and will be referred to as harmonized motor scores or motor scores throughout this document. This metric was generated after two stages. For each individual observation: (1) The UPDRS and MDS-UPDRS Part III sub-items scores were summed to generate the Part III subtotal score; and (2) The UPDRS Part III subtotal score was transformed to the respective MDS-UPDRS Part III score to yield the harmonized motor scores (refer to section below). The transformation of the individual UPDRS Part III subtotal score to the respective MDS-UPDRS relied on a previously derived formula based on a Hoehn and Yahr stage I or II (76) (Equation 1):

$$\mathrm{MDS-UPDRS_{III}} = \mathrm{UPDRS_{III}} \times 1.2 + 2.3 \quad \text{(E1)}$$

**Statistical Model**

The rate of progression on the harmonized motor scores was compared between subjects with a scan without evidence of DAT deficit (SWEDD or biomarker negative) and those with DAT deficit (biomarker positive) using a generalized linear mixed-effects model (12). An unstructured covariance matrix was estimated. The calculated probabilities (P values) were generated via an F-test based on the Kenward and Roger approach (13). The following hypotheses were tested at one-tailed $\alpha$ of 0.05:

- Null hypothesis ($H_0$): The fixed effect of interaction between biomarker status (SWEDD) and time is equal to or greater than zero (i.e., the SWEDDs progression rate is equal to or greater than that of DAT deficit subjects).
- Alternative hypothesis ($H_a$): The fixed effect of interaction between biomarker status (SWEDD) and time is less than zero.

**Fixed and Random Effects**

Pre-specified fixed effects were study, time, biomarker status, and interaction between biomarker status and time. The pre-specified mixed-effects model is represented in Conrado et al. (14). For comparison, a model without any adjustment for biomarker status was also fitted (i.e., reduced model). The fixed effect of interaction between study, and biomarker status and time was explored to compare progression rates between studies. Given the neurodegenerative nature of the PD, the fixed effect of age was also explored. Pre-specified random effects were subject within study and measurement error. The random effect of subject within study was incorporated in intercept and time. Model selection criteria and evaluation of model performance are described in Conrado et al. (14).

**Comparison of Magnitude of Motor Scores Worsening between Biomarker Categories**

The magnitude of worsening of motor scores was compared in subjects with DAT deficit and SWEDD subjects based on a previously published study which highlights a data driven strategy outlining the definition of meaningful clinical change using existing outcome measures (15). In that study, a cross-sectional analysis to identify the clinically important difference (CID) for UPDRS was performed using a distribution and an anchor-based approach. The study data was from 653 subjects diagnosed with PD who underwent routine UPDRS office assessments for 41 months. The authors estimated a minimal CID in the UPDRS Part III of 2.5 points (15). Applying the aforementioned conversion formula (16) to translate such difference to the MDS-UPDRS Part III, we have a minimal CID of 3 points.

Using the previously mentioned 41 months as a reference time (15), under the assumption of a linear progression of the harmonized motor scores during this time period:

- The estimated fixed effect of time (i.e., progression rate for subjects with DAT deficit) was multiplied by 41 months to yield the average magnitude of worsening (i.e., change from baseline) in the motor scores for DAT deficit subjects.
- The estimated fixed effect of the interaction between biomarker status and time (i.e., average rate of progression in subjects with DAT deficit subtracted from the average rate of progression in SWEDDs) was multiplied by 41 months. This yielded the average difference in the magnitude motor scores worsening (i.e., change from baseline) between biomarker statuses.
- The sum of the estimated fixed effect of time and the estimated fixed effect of the interaction between biomarker status and time yielded the average rate of progression in SWEDD subjects. This was multiplied by 41 months to yield the average magnitude of worsening (i.e., change from baseline) in the motor scores for SWEDD subjects.

The 90% confidence intervals (CIs) for the above quantities (from the parametric bootstrap) were also multiplied by 41 months to yield the respective confidence ranges. The aforementioned calculations were also performed for 24 months given the scope of this analysis.

**Identification of Subjects Who Experience a Clinically Important Worsening of the Motor Scores**

We sought to compare the early stage PD criteria + DAT imaging versus the early stage PD criteria alone regarding the ability to identify subjects who experience a CID.

The harmonized motor scores at time 0 (baseline) and 41 months were predicted for each subject.

The 0-month score was subtracted from the 41-month score to yield the individual change from baseline difference. The number of subjects with a difference equal to or greater than 3 points (i.e., CID) was summarized for the analysis dataset. From the subjects with a CID:

- The number of subjects with DAT deficit was calculated to yield the ability of the DAT imaging to identify patients who experience a CID.
- The number of SWEDD subjects was calculated to yield the proportion of subjects who experience a CID and would be excluded in a DAT-based enriched trial enrolling only DAT deficit subjects.

The aforementioned calculations were also performed for 24 months given the scope of this analysis.

Clinical Trial Simulations and Statistical Power Analyses

Monte Carlo-based clinical trial simulations were performed to compare the statistical power by sample size in trials with and without DAT imaging enrichment. Enriched trials had only subjects with DAT deficit, whereas non-enriched trials also included 15% SWEDD subjects (17).

Two thousand placebo-controlled clinical trials with and without enrichment were simulated using the fixed and random effect parameter values from the chosen model for a PRECEPT-like study. The trials size ranged from 100 to 700 subjects per arm with duration of 24 months. A hypothetical drug effect of 50% reduction in the disease progression rate was simulated for subjects with DAT deficit in the drug arms.

For each simulated trial, a linear mixed-effects model was fitted and P values were calculated as in Statistical Model. Fixed effects and random effects were as in the chosen model except the fixed effect of biomarker status and its interaction with time were not accounted for in the analyses. The power, namely the probability of detecting the drug effect, was calculated as the proportion of trials for which the parameter estimate for the interaction between time and treatment showed a beneficial drug effect with a two-tailed P value less than 0.05.

**Supplementary Statistical Analyses Investigating Baseline Scores and DAT Imaging Status as Predictors of Progression Rate**

A supplementary statistical analysis was performed to investigate the effect of baseline scores and DAT imaging status on progression rate.

Because the distribution of observed baseline motor scores shows some degree of overlap in the baseline scores between SWEDD and DAT-deficient subjects (Figure 1), a baseline-matched subset of the data was created for subsequent use. In this baseline-matched subset, DAT deficit subjects were included only if there was more than one SWEDD subject with the same observed baseline score (rounded to zero decimal places); likewise, SWEDD subjects were included only if there was more than

one DAT deficit subject with the same observed baseline score (rounded to zero decimal places). Given the association between biomarker status and baseline motor scores, a baseline-matched dataset decreases the likelihood of confounding effects, and helps investigate the separate contribution of baseline and biomarker status on the rate of progression.

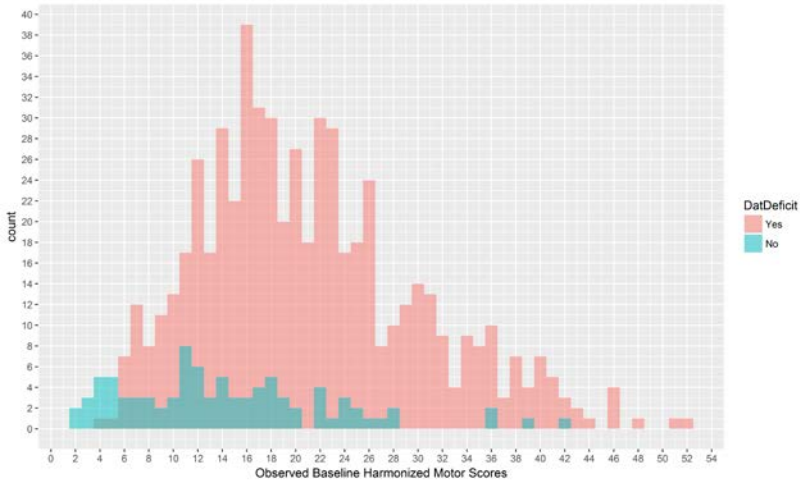


Figure 1 Histogram of observed baseline harmonized scores (number of subjects according to their baseline harmonized motor score)

Using the 'baseline-matched' subset, a supplementary statistical analysis was conducted to explore the effect of baseline score on the rate of progression. In this analysis, the model structure was identical to the final model, except that effect of baseline on progression rate (fixed effect) was also included.

A sensitivity analysis was also performed using the entire analysis dataset. In this analysis, the model structure was identical to the final model, except that the following were included: effect of biomarkers status SWEDD on progression rate, effect of baseline on progression rate, and additional effect of baseline on progression rate in SWEDDs.

**Results**

The results presented in this section, except by the supplementary analyses, have recently been published:

- Conrado DJ, Nicholas T, Tsai K, et al., Dopamine Transporter neuroimaging as an enrichment biomarker in early Parkinson's disease clinical trials: a disease progression modeling analysis. Clinical and Translational Science, 2017 Jul 27. doi: 10.1111/cts.12492. [Epub ahead of print]
- Available at https://www.ncbi.nlm.nih.gov/pubmed/28749580

**Data Summary**

The analysis dataset (i.e., after data exclusion) included a total of 672 subjects diagnosed with early stage PD and a total of 4521 observations in the (baseline, 25 months) interval. Unscheduled visits with known time in the (baseline, 25 months) interval were also included. There were 6 subjects with missing biomarker status who were not included in the analysis dataset. Other exclusions occurred at the visit level and reasons are listed in Conrado et al. (14).

Subjects' baseline demographics and clinical characteristics stratified by study are summarized in Table 1. Subjects were between the ages of 31 and 84 years with a mean age of approximately 60 years in both studies. The majority of subjects in each study were male with DAT deficit. The proportion of SWEDD subjects in the analysis dataset was 13% and 14% for PPMI and PRECEPT, respectively. The

mean harmonized motor scores at baseline of approximately 20 points were similar for both studies. The time course of the mean observed harmonized motor scores is presented in Figure 2.

Table 1 Baseline characteristics by study

| Baseline | PPMI | PRECEPT |
|---|---|---|
| Sample size | 481 | 191 |
| Sex, % | Female (35), Male (65) | Female (34), Male (66) |
| Age in year, mean (range) | 61 (33, 84) | 59 (31, 84) |
| DAT deficit, % | Yes (87), No (13) | Yes (86), No (14) |
| Harmonized motor scores, mean (range) | 20 (2, 51) | 21 (5.3, 52) |

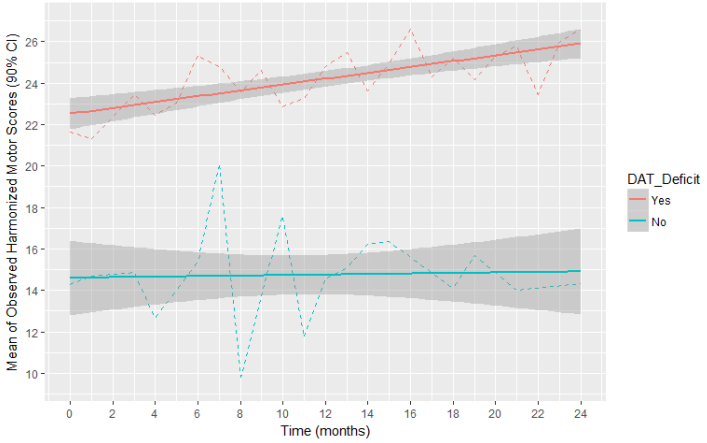


Figure 2 Observed harmonized motor scores.

Dotted lines are mean of observed scores binned by month; bins with less than 15 records were not plotted. The solid lines are linear smooths, and the shaded areas are the respective 90% confidence intervals (CIs).

**Linear Mixed-Effects Model**

A linear mixed-effects model, with an error distribution of Gaussian shape and an identity link function, was utilized to compare the rate of progression on the harmonized motor scores between subjects with DAT deficit and SWEDDs.

In the full model, fixed and random effects were as described in Fixed and Random Effects with an additional fixed effect of age. The fixed effect of interaction between age and time was not significant and not included in the model. Likewise, the fixed effect of interaction between study, and biomarker status and time was not statistically significant and not included in the model. In the reduced model, fixed and random effects were as in the full model, except that the fixed effect of biomarker status and the fixed effect of interaction between biomarker status and time were not included. The R code, output summary and analysis of variance (ANOVA) table for reduced model, full model and model comparison can be found in Conrado et al. (14).

Full model diagnostics suggest an adequate fit of the longitudinal changes in the harmonized score (14). The Akaike information criterion (AIC) for the reduced and full model were 29713.22 and 29637.17, respectively, indicating improvement when considering biomarker status. Additional statistics on the comparison between models can be found in Conrado et al. (14). A sensitivity analysis was conducted by fitting the full model with the harmonized motor scores in the natural logarithm and

logit domains. These transformations did not improve the heteroscedasticity, yielding increased Pearson residuals for the lower scores as compared to those for the higher scores.

The population predicted harmonized motor scores over time are presented in Figure 3. The parameter estimates for the full model with their 90% CI from the bootstrap are presented in Table 2. Noteworthy:

- The estimated fixed effect of interaction between biomarker status and time was -0.13 (90% CI: -0.23, -0.04) point/month for SWEDDs (one-tailed P value = 0.01). This means that SWEDD subjects have an average monthly progression in the harmonized motor scores that is 0.05 (90% CI: -0.04, 0.13) point/month or 0.13 point/month lower than that in subjects with DAT deficit (0.18 point/month; 90% CI: 0.14, 0.21).
- The estimated fixed effect of biomarker status was -7.69 (90% CI: -9.4, -6.04) points for SWEDD subjects; hence, SWEDDs have an average baseline harmonized motor score that is 7.69 points lower than those with DAT deficit.
- The fixed effect of age was estimated as 0.19 (90% CI: 0.14, 0.24) point, which means that, on average, the baseline harmonized motor score increases by 0.19 point for each year of age. Thus, the baseline score for a typical 60-year subject with DAT deficit is expected to be 21.54 points.

Table 2 Parameter estimates with 90% confidence intervals (CI) from parametric bootstrap

| Parameter | Interpretation | Estimate | CI |
|---|---|---|---|
| Intercept (points) | Baseline | 10.08 | 6.83, 13.61 |
| Study PRECEPT | Effect of PRECEPT study on baseline | 1.20 | 0.01, 2.34 |
| Age | Effect of year of age on baseline | 0.19 | 0.14, 0.24 |
| No DAT deficit | Effect of absence of DAT deficit on baseline | -7.69 | -9.4, -6.04 |
| Time (point/month) | Slope or rate of change | 0.18 | 0.14, 0.21 |
| Interaction time and no DAT deficit | Effect of absence of DAT deficit on slope | -0.13 | -0.23, -0.04 |
| Subject effect on baseline | Variance of random effects | 73.36 | 65.63, 81.35 |
| Subject effect on slope | Variance of random effects | 0.16 | 0.13, 0.18 |
| Measurement error (points) | Standard deviation | 4.72 | 4.63, 4.81 |

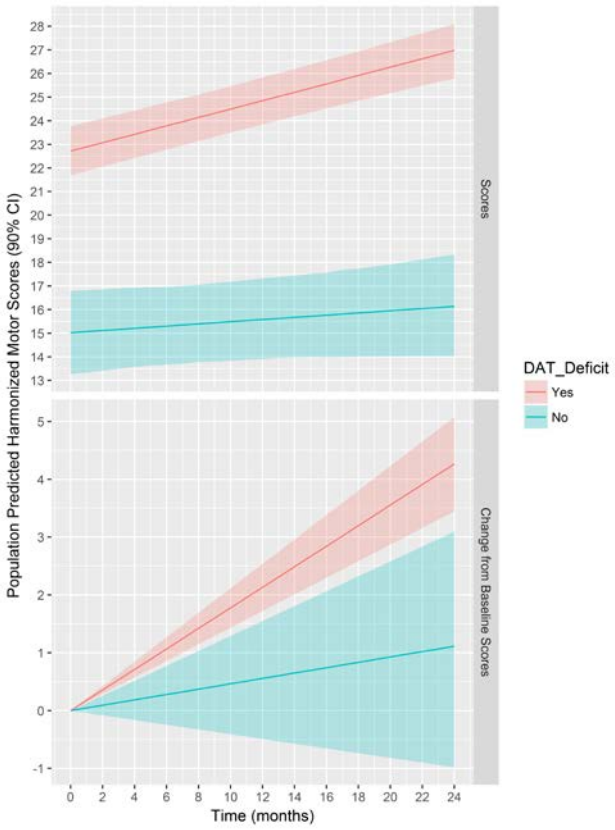


Figure 3 Population predicted harmonized motor scores.

Shaded area is the 90% confidence interval (CI) from bootstrap. Predictions are for a PRECEPT-like study with average age of 60 years old.

**Magnitude of Motor Scores Worsening between Biomarker Conditions**

The magnitude of motor scores worsening (i.e., change from baseline at 24 and 41 months) in DAT deficit and SWEDD subjects was compared. As aforementioned, 41 months was based on the previously published study (15) under the assumption of a linear progression rate.

The change from baseline of the motor scores at 41 months was 7.31 (90% CI: 5.89, 8.68) and 1.91 (90% CI: -1.68, 5.29) points for subjects with DAT deficit and SWEDDs, respectively. The average difference in the change from baseline score at 41 months between biomarker statuses was -5.41 (90% CI: -1.64, -9.25) points. This difference indicates that subjects with DAT deficit have an average of 5.41 points higher (worse) change from baseline score at 41 months than SWEDDs, which is greater than the minimal CID of 3 points.

The change from baseline of the motor scores at 24 months was 4.28 (90% CI: 3.45, 5.08) and 1.12 (90% CI: -0.98, 3.1) points for subjects with DAT deficit and SWEDDs, respectively. The average difference in the change from baseline score at 24 months between biomarker statuses was -3.16 (90% CI: -0.96, -5.42) points. This difference indicates that subjects with DAT deficit have an average of 3.16 points higher (worse) change from baseline score at 24 months than SWEDDs, which is greater than the minimal CID of 3 points.

**Subjects who experience a clinically important worsening of the motor scores**

The predicted individual change from baseline difference in the harmonized motor scores at 24 and 41 months was used to determine the subjects with a CID (i.e., difference equals to or greater than 3 points). From the subjects with a CID:

- The number of subjects with DAT deficit was calculated to yield the ability of the DAT imaging to identify patients who experience a CID.
- The number of SWEDD subjects was calculated to yield the proportion of subjects who experience a CID and would be excluded in a DAT-based enriched trial enrolling only DAT deficit subjects.
- At 41 months:

  Of the 672 subjects diagnosed with early stage PD in the analysis dataset, 420 subjects were estimated to experience a CID or a clinically important worsening of the harmonized motor scores. Of the 420 CID subjects, 387 had DAT deficit and 33 were SWEDDs. This means that the ability of the DAT imaging to identify subjects who experience a CID is 92.14%. Conversely, of the 420 CID subjects, 7.86% would be excluded in a DAT-based enriched trial enrolling only DAT deficit subjects.

- At 24 months:

  Of the 672 subjects diagnosed with early stage PD in the analysis dataset, 368 subjects were estimated to experience a CID or a clinically important worsening of the harmonized motor scores. Of the 368 CID subjects, 340 had DAT deficit and 28 were SWEDDs. This means that the ability of the DAT imaging to identify subjects who experience a CID is 92.39%. Conversely, of the 368 CID subjects, 7.61% would be excluded in a DAT-based enriched trial enrolling only DAT deficit subjects. These results are summarized in Figure 4.

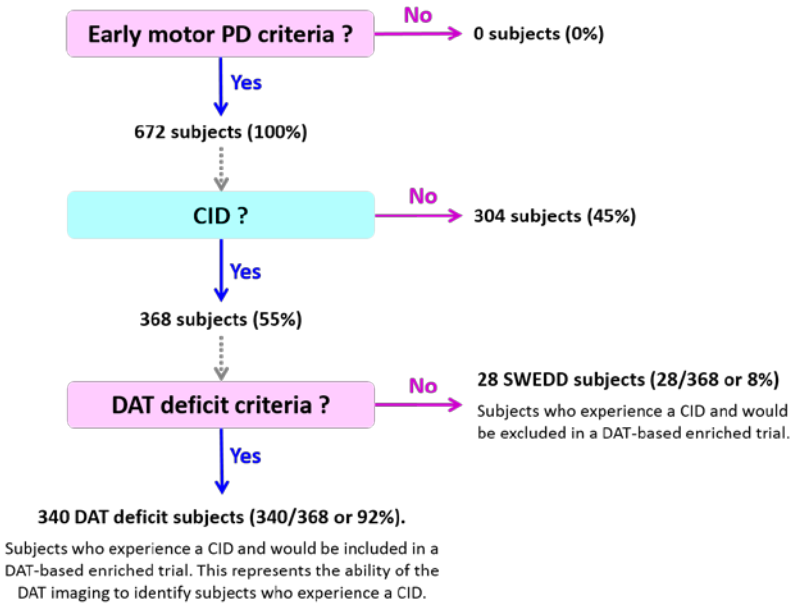


Figure 4 Ability of DAT imaging to identify subjects who experience a clinically important worsening of the harmonized motor scores.

Clinically important worsening or CID was defined as change from baseline in the harmonized motor scores of at least 3 points at 24 months. A DAT-based enriched trial is one that includes only DAT deficit subjects. Solid arrows mean that criteria are being applied.

## Clinical trial simulations and statistical power

Clinical trial simulations were performed to compare the statistical power by sample size in trials with and without DAT imaging enrichment.

Two thousand placebo-controlled clinical trials with and without DAT imaging enrichment were simulated utilizing the Monte Carlo technique. The trial size ranged from 100 to 700 subjects per arm. A hypothetical drug effect of 50% reduction in the disease progression rate was simulated for subjects with DAT deficit in the drug arm. As observed in the data, this simulation example captures a small proportion of SWEDDs who show motor progression. From the simulated enriched and non-enriched trials, the median harmonized motor scores over time for a 600-subject per arm trial size of 24 months is presented in Figure 5.

The statistical power is the probability of detecting an existent effect, in this case, the drug effect of 50% reduction in the disease progression rate. The estimated power by sample size graph for DAT imaging enriched (i.e., only subjects with DAT deficit) and non-enriched (i.e., 15% SWEDD subjects) trials is presented in Figure 6. Based on the simulations, interpolation shows that approximately 475 subjects per arm would be required in a non-enriched placebo-controlled clinical trial in order to detect a drug effect of 50% reduction in the progression rate with a 80% probability (type II error or $\beta$ = 0.20 (18)) at $\alpha$ = 0.05. Conversely, the same 80% probability of detecting an analogous drug effect at $\alpha$ = 0.05 is achieved with approximately 355 subjects per arm in an enriched clinical trial. This represents a reduction in sample size of approximately 24%. Naturally, this enrichment magnitude will vary, depending on the nature of the clinical trial designed being considered, the assumptions for drug effect magnitude, and the nature of the hypothesis being tested.

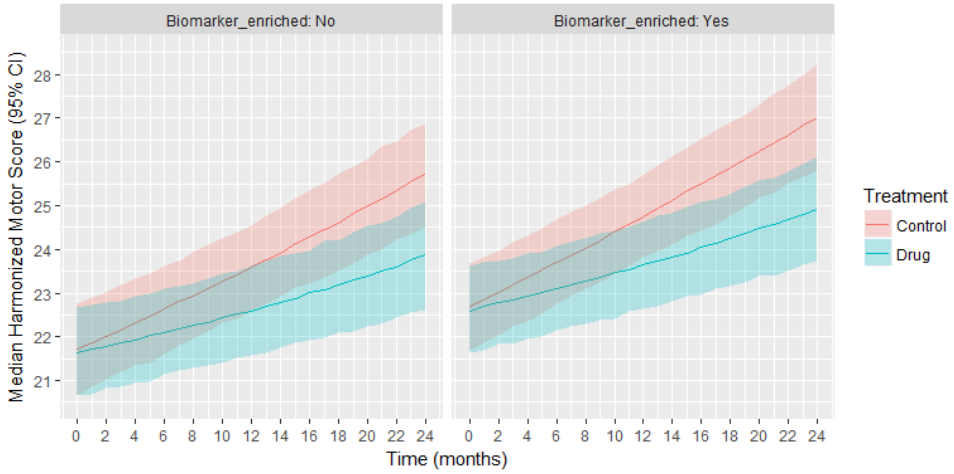


Figure 5 Simulated placebo-controlled clinical trials without and with DAT imaging enrichment.

600 subjects per arm and a hypothetical drug effect of 50% reduction in the progression rate of subjects with DAT deficit (N = 2,000 simulations). Shaded area is the 95% inter-percentile range (CI) for the collection of median scores from the simulations.

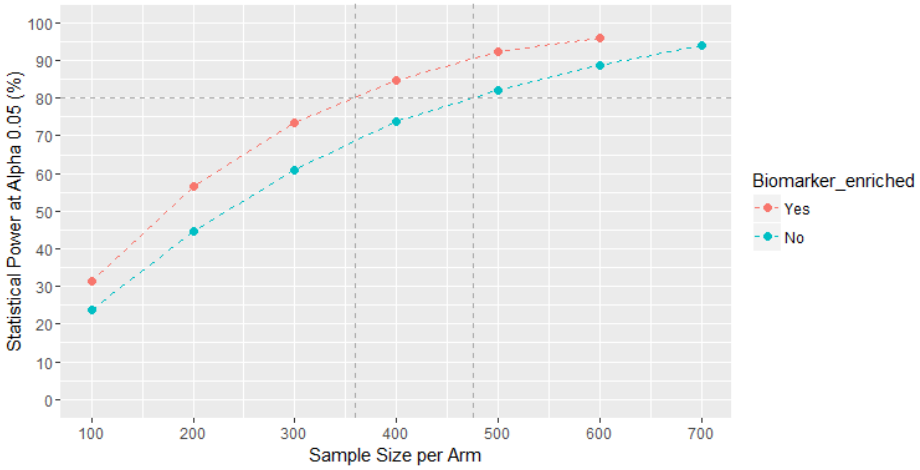


Figure 6 Statistical power by sample size for placebo-controlled DAT imaging enriched and non-enriched clinical trials with a drug effect of 50% reduction in the progression rate

**Supplementary statistical analyses investigating baseline scores and dat imaging status as predictors of progression rate**

Table 3 shows the results of the supplementary analysis using the 'baseline-matched' subset. The estimated fixed effect of interaction between biomarker status and time of -0.19 point/month for SWEDDs remained statistically significant (two-tailed P-value < 0.01), even after the fixed effect of interaction between baseline and time has been accounted for.

Table 3 Parameter estimates from the supplementary analysis using the 'baseline-matched' subset (N = 463)

| Parameter | Estimate | *P*-value |
|---|---|---|
| Intercept at baseline scores (points) | 12.55 | * |
| Effect of PRECEPT on baseline | 0.59 | NS |
| Effect of year of age on baseline | 0.08 | * |
| Effect of SWEDD on baseline | -2.41 | * |
| Slope or progression rate (point/month) | -0.19 | * |
| Effect of SWEDD on progression rate | -0.19 | * |
| Effect of baseline on progression rate | 0.03 | * |

* Indicates two-tailed P-value lower than 0.01; NS indicates two-tailed P-value greater than 0.05.

Table 4 shows the results of the supplementary analysis using the entire dataset. The estimated fixed effect of interaction between biomarker status and time of -0.24 point/month for SWEDDs remained statistically significant (two-tailed P-value < 0.05), even after the fixed effect of interaction between baseline and time as well as time, baseline score and biomarker status have been accounted for.

Table 4 Parameter estimates from the supplementary analysis using the entire dataset

| Parameter | Estimate | *P*-value |
|---|---|---|
| Intercept at baseline scores (points) | 12.71 | * |
| Effect of PRECEPT on baseline | 0.79 | NS |
| Effect of year of age on baseline | 0.15 | * |
| Effect of SWEDD on baseline | -7.70 | * |

| | | |
|---|---|---|
| Slope or progression rate (point/month) | -0.31 | * |
| Effect of SWEDD on progression rate | -0.24 | * |
| Effect of baseline on progression rate | 0.02 | * |
| Additional effect of baseline on progression rate in SWEDD | 0.02 | * |

* Indicates two-tailed P-value lower than 0.05; NS indicates two-tailed P-value greater than 0.05.

These results demonstrate that SWEDD status is an independent predictor of motor progression of PD, independent of baseline severity.

**Summary and conclusion**

The evidence in the field of PD at present suggests that SWEDD indicates a high likelihood of an absence of neurodegeneration in a subject with suspected Parkinson's disease symptoms.

- The rate of SWEDD subjects in the PPMI observational cohort is >15%.
- There is a poor levodopa response in SWEDD subjects (19) (20).
- A significant percent of cases defined as SWEDD are seen in several trials of PD where DAT imaging has been employed. Specifically, published reports range from 3-15% SWEDD in clinical trials to date (21) (22) (1) (23) (24).
- Evaluation of the incidence of SWEDD subjects as a function of the duration of disease diagnosis in different PD clinical trials demonstrates that the rate of SWEDD is greater at earlier stages of PD.
- The early motor stage of PD aligns with the clinical trial populations being targeted for ongoing and future therapeutic trials (25).
- Patients that are identified as SWEDD at baseline when followed by sequential dopaminergic imaging and clinical evaluation show a lack of disease progression as well as lack of conversion from SWEDD to DAT deficiencies (26) (27) (28).
- Image acquisition variations do not account for the results on disease progression differences between SWEDD and DAT-deficient subjects in both PPMI and PRECEPT.

In this context, the objective of this work was to evaluate DAT neuroimaging as an enrichment biomarker in clinical trials targeting early stage PD.

Individual longitudinal data of subjects diagnosed with early stage PD in the PPMI observational study (PD and SWEDD cohorts) and in the PRECEPT clinical trial (placebo arm) were utilized in this analysis. The analysis dataset had a total of 672 PD subjects and a total of 4521 observations in the (baseline, 25 months) interval. The presented subject's baseline demographics and clinical characteristics were similar in both studies. The dependent variable was the harmonized motor scores in that PRECEPT and PPMI used the UPDRS and the MDS-UPDRS assessment scales, respectively. The percentage of ineligible screened patients due clinical reasons was approximately 11% in PRECEPT, (Appendix, Figure A2). In turn, the proportion of SWEDD subjects in the analysis dataset was 13% and 14% for PPMI and PRECEPT, respectively. Such a proportion is also the percentage of patients expected to be ineligible in a DAT-based enriched trial enrolling only DAT deficit subjects due to biomarker status.

The rate of worsening in the motor scores were compared between SWEDD and DAT deficit subjects using a linear mixed-effects model testing the following hypotheses at one-tailed $\alpha$ of 0.05: (a) $H_0$, the fixed effect of interaction between biomarker status (SWEDD) and time is equal to or greater than zero

(i.e., the SWEDDs progression rate is equal to or greater than that of DAT deficit subjects); (b) $H_a$, the fixed effect of interaction between biomarker status (SWEDD) and time is less than zero. The estimated fixed effect of interaction between biomarker status and time was -0.13 (90% CI: -0.23, -0.04) point/month for SWEDD subjects (one-tailed P value = 0.01). This result suggests that SWEDDs have an average monthly progression in the harmonized motor scores that is 0.05 point/month or 0.13 point/month lower than those with DAT deficit (0.18 point/month). The fixed effect of interaction between study, and biomarker status and time was not statistically significant suggesting that the rate of progression of subjects with DAT deficit and SWEDD subjects are comparable between PPMI and PRECEPT.

Supplementary statistical analyses investigating the effect of baseline scores and DAT imaging status on progression rate showed that the fixed effect of interaction between biomarker status and time remained statistically significant, even after the fixed effect of interaction between baseline and time has been accounted for. The estimated fixed effect of interaction between biomarker status and time was -0.19 and -0.24 point/month for SWEDDs in the supplementary analysis using the baseline-matched dataset and the entire dataset, respectively. Consistent estimates and statistical significance of the effect of SWEDD status on progression rate across original and supplementary analyses constitutes persuasive evidence that the average lower progression rate in SWEDD subjects is not simply due to their lower baseline scores.

The magnitude of worsening in the motor scores was compared between SWEDD and DAT deficit subjects. The change from baseline of the motor scores at 24 months was 4.28 (90% CI: 3.45, 5.08) and 1.12 (90% CI: -0.98, 3.1) points for subjects with DAT deficit and SWEDDs, respectively. The average difference in the change from baseline score at 24 months between biomarker statuses was -3.16 (90% CI: -0.96, -5.42) points. Such difference indicates that subjects with DAT deficit have an average of 3.16 points higher (worse) change from baseline at 24 months score than SWEDDs, which is greater than the minimal CID of 3 points.

An individual-based analysis identified the number of early stage PD subjects with a CID at 24 months. Out of the 672 PD subjects, 368 subjects were estimated to experience a CID. Among those, the proportion of subjects with DAT deficit was 92.39%, which represents the ability of the DAT imaging to identify patients who experience a CID. Conversely, of the 368 CID subjects, 7.61% were SWEDDs, which means that an acceptable fraction of CID subjects would be excluded by DAT imaging. This can be considered as a positive feature of an enrichment biomarker. Sensitivity, specificity and predictive values lack practical utility in this context because the scope herein is not diagnostic, but is the use of DAT imaging as an enrichment biomarker and a statistically significant predictor of disease progression.

Clinical trial simulations comparing the statistical power by sample size in trials with and without DAT imaging enrichment showed that exclusion of SWEDD subjects allowed a meaningful reduction of trial size, while maintaining adequate statistical power. In the illustrated simulation herein, enriched trials had only subjects with DAT deficit, whereas non-enriched trials also included 15% SWEDD subjects (17). A drug effect of 50% reduction in the disease progression rate was simulated for subjects with DAT deficit. To achieve a statistical power of 80% (i.e., 80% probability of detecting the drug effect) at $\alpha$ = 0.05, approximately 475 and 360 subjects per arm would be required in a non-enriched and enriched placebo-controlled clinical trial, respectively. This represents a reduction in sample size of approximately 24%. This enrichment magnitude will vary, depending on the nature of the clinical trial designed being considered, the assumptions for drug effect magnitude, and the nature of the hypothesis being tested.

In conclusion, analysis of integrated data from independent observational and RCT shows that SWEDD subjects have a significant difference in rate of progression as compared to those subjects with DAT-deficiency at baseline. Exclusion of SWEDD subjects allows a meaningful reduction of the trial size. Collectively, these findings imply that a SPECT finding of functional integrity of presynaptic dopaminergic terminals in a case of suspected PD is associated with a good prognosis, whatever the ultimate diagnosis. Exclusion of cases of SWEDD from future clinical trials will improve the chance of determining clinical benefit of new drug candidates for patients with PD.

**CHMP Qualification opinion**

Dopamine Transporter Neuroimaging is qualified to be used as an enrichment biomarker in Parkinson's disease clinical trials targeting early motor stages of the disease.

Identifying patients with early motor deficits in conjunction with confirming reduction of DAT levels, as measured by SPECT neuroimaging, is a useful means of selecting subjects for clinical trials. It is envisioned that the biomarker can help predict which individuals will have negligible progression rates, subjects defined as scans without evidence of dopamine deficiency (SWEDD), and which individuals will have detectable and clinically-relevant progression rates over the course of clinical trials of up to two years in duration. It should be noted that the qualification opinion does not mandate the use of DAT imaging or exclude the possibility of the use of alternative methods in the confined application.

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# APPENDIX

## CONTEXT OF USE

The following flow diagram illustrates the proposed outline for sponsors to employ in clinical trials using the neuroimaging biomarker to aid in subject selection for the defined target population:

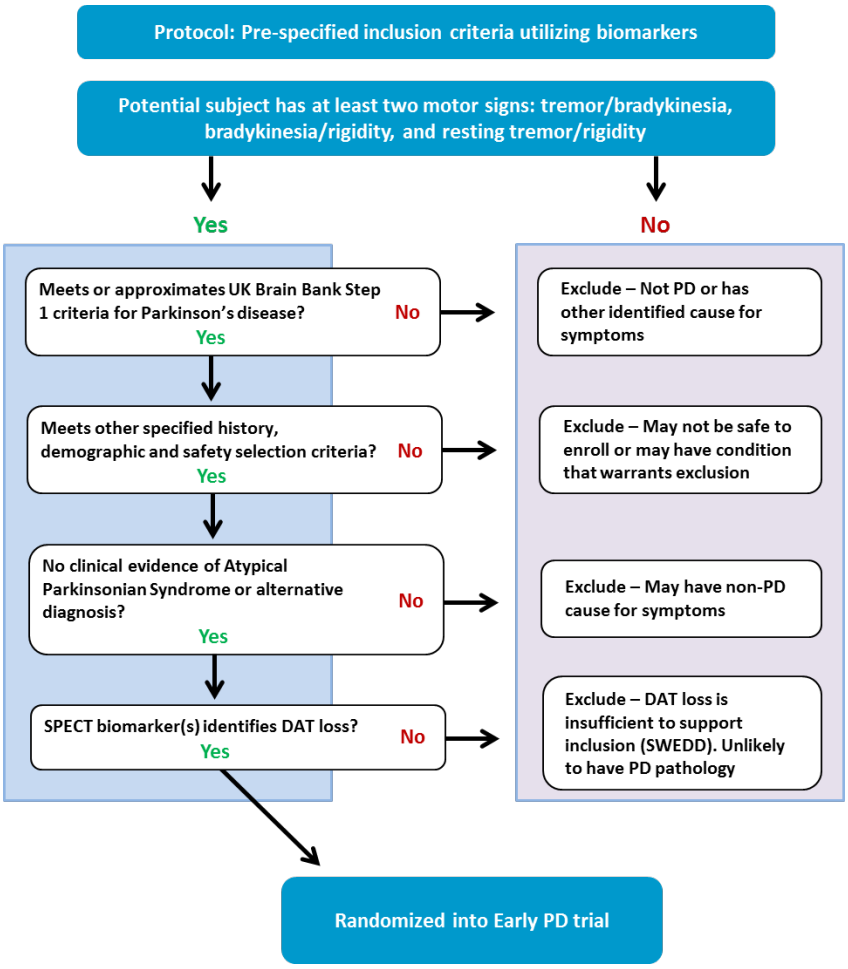



Figure A1 Proposed flow chart for the application of DAT imaging as an enrichment biomarker in clinical trials of patients with motor signs of early PD

## Data sources

### Important Comparative Aspects of the Data Sources

PRECEPT and PPMI represent uniquely rich cohorts of well characterized subjects with early stage (de novo) PD where subject-level data is available to CPP for analyses to support regulatory science goals. Both studies include similar patient populations from multicenter global sites with application of DAT imaging at baseline and long term clinical follow up. The use of both observational and randomized clinical trial (RCT) populations aides in the confidence of predictability of the results to prospective trial populations that align with the proposed context-of-use. Notably, differences in these studies exist including the use of distinctive but chemically related imaging ligands and the use of two distinct but related outcome measures (UPDRS vs. MDS-UPDRS).

Additional parameters worth noting include the imaging methodologies used at baseline as well as the duration of clinical follow up. The two studies used DAT imaging at baseline for distinctive purposes. Specifically, PPMI applied visual reads of DAT SPECT scans using 123I-ioflupane at baseline as a criterion for subject selection and recruitment into defined classifications of subjects (i.e., de novo PD vs. SWEDD). PRECEPT applied quantitative measures of DAT levels using β-CIT SPECT imaging to all subjects at both baseline and follow up. All subjects in PRECEPT were randomized into either placebo or CEP 1347 treatment arms, independent of DAT levels, and identification of the subject status as SWEDD was carried out at the conclusion of the study.

Both clinical PD populations include relatively long duration follow up. The duration of follow up was three years in PRECEPT and is still ongoing in PPMI. At present, PPMI has four year follow up data available for analyses with planned discontinuation in 2018 (year 6). PRECEPT subjects have been followed for longer duration in the PostCEPT study (27). Remarkably, two thirds of the PRECEPT subjects agreed to be included in PostCEPT for a duration of three years.

SWEDD subjects in PPMI learned their imaging results after the baseline scans were interpreted. Subjects were offered the opportunity to remain enrolled in PPMI to advance the understanding of PD and all opted to continue. In PRECEPT, all subjects and clinicians remained blinded throughout the duration of the clinical trial. SWEDD status was not defined in PRECEPT until study completion. Therefore, all PRECEPT subjects were blinded until study completion. Finally, traditional placebo effect due to treatment expectation (as in a clinical trial) should not be expected in PPMI.

Table A1 PRECEPT CEP-1347; Study C1347c/204/PD/US-CA

**Study design and dates:** Phase 2/Phase 3, multicenter, randomized, double-blind, placebo-controlled, dose-finding study to determine if treatment with CEP-1347 delays the time to onset of disability sufficient to require dopaminergic therapy in patients with early Parkinson's disease who currently do not receive or require dopaminergic therapy for symptomatic control of their disease. Planned treatment duration was a minimum of 24 months. Visits one-month and three-months after start of treatment and approximately every three months thereafter. The study was discontinued prior to completion due to futility (see Parkinson's Study Group, 2007). A total of 108 of 191 subjects randomized to placebo (57%) had reached the primary point of disability requiring dopaminergic therapy compared with active CEP1347: 133 of 205 on 10mg, 126 of 212 on 25 mg, and 127 of 198 on 50 mg.

**Main inclusion criteria**: Age 30 years or older at time of diagnosis of Parkinson's disease; idiopathic Parkinson's disease with at least 2 cardinal signs of disease: resting tremor, bradykinesia, or rigidity; Modified Hoehn and Yahr stage <= 2.5.

**Main exclusion criteria**: Atypical Parkinsonism due to drugs, metabolic disorders, encephalitis, or other neurodegenerative diseases; confirmed diagnosis of Parkinson's disease for more than five years; tremor score of three or more in any body part; Mini-Mental State Exam (MMSE) score <= 26; Beck depression score >= 15; treatments within 60 days with potentially confounding anti-Parkinson's disease effects; treatments within six months that may induce Parkinson's disease; treatments within 28 days with specified substrates for Cytochrome P450 3A4/5 (CYP3A4/5) and inhibitors of CYP3A4/5.

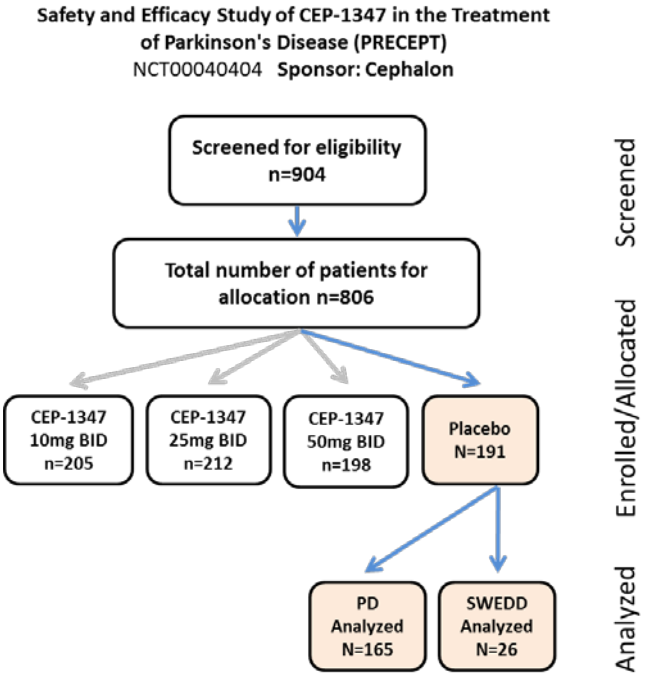
**Primary endpoint:** Time to onset of disability sufficient to require dopaminergic therapy.
**Secondary endpoints**: Rate of change from baseline in total UPDRS score (Parts I - III) at time of onset of disability sufficient to require dopaminergic therapy; change from baseline in total UPDRS score (Parts I - III) at 24-months; [$^{123}$I]β-CIT SPECT imaging: percent change in mean striatal uptake from baseline to 24 months; [$^{123}$I]β-CIT SPECT imaging: percent change in ipsilateral striatum, contralateral striatum, mean caudate, ipsilateral caudate, contralateral caudate, mean putamen, ipsilateral putamen, contralateral putamen uptake; rate of change from baseline in Schwab and England Activities of Daily Living (S&E-ADL) scale at the time of onset of disability sufficient to require dopaminergic therapy.

**Determination of primary endpoint**: The specific quantified endpoint is the date on which the investigator determines the patient has reached a level of disability sufficient to require initiation of dopaminergic therapy. Four prescribed criteria guide this determination (29):
impairment in gait and balance
threat to part or full time employment (if applicable)
threat to domestic capabilities
functional impairment in self-care skills

**Statistical analysis of primary endpoint:** The null hypothesis is that the hazard rate, which is assumed to be constant across all study months, is identical in the four treatment groups (10, 25, and 50 mg *bis in die* (BID) of CEP 1347 and placebo). This is tested by an overall logrank test applied to compare all two treatment groups. If this is significant at alpha equal to 0.05, pairwise comparisons of each CEP 1347 dose to placebo are made using a two-tailed, logrank test at alpha equal to 0.05.

**Follow up:** Dr. Ken Marek, Institute of Molecular Neuroimaging, has agreed to provide data from the longer term follow up from PRECEPT including DAT β-CIT imaging data from the following subjects: 800 baseline, 700 22 month, 500 50 month and 400 72 month scans.

763 References: (2) (29) (22)




764

765 Figure A2 Schema for the safety and efficacy study of CEP-1347 in the treatment of Parkinson's
766 disease (PRECEPT).

767 All individuals in the control arm (n=191) had baseline SWEDD status data, of which 165 were part of
768 the PD cohort and 26 were part of the SWEDD cohort.

769 Table A2 Parkinson's Progression Markers Initiative (PPMI)

**Study design and dates:** The PPMI is a multicenter observational trial supported by a consortium of academic centers, Parkinson's disease foundations, and pharmaceutical and biotechnology companies to collectively design, fund, and implement a comprehensive research program to identify and validate markers of Parkinson's disease progression **(2)**. This effort is modeled after the Alzheimer's Disease Neuroimaging Initiative (ADNI) **(30)**, examining progression markers in patients with predementia stages of Alzheimer's disease. The primary objective of PPMI is to identify clinical, imaging and biologic markers of PD progression for use in clinical trials of disease-modifying therapies. The specific aims to accomplish the primary objective are:

Establish standardized protocols for acquisition, transfer and analysis of clinical, imaging and genomic data $t_{ha}t$ can be used by the PD research community.

Develop a comprehensive and uniformly acquired clinical and imaging dataset and biological samples.

Investigate existing and identify novel clinical, imaging, and genomic Parkinson disease progression markers to identify quantitative individual measures or combination of measures that demonstrate interval change in PD patients in comparison to healthy controls or in sub-sets of PD patients defined by baseline assessments, progression milestones and/or rate of clinical, imaging, or genetic change.

Conduct preliminary verification studies on promising biological markers using stored collected samples.

The study aimed at the onset to enroll 600 subjects (400 *de novo* Parkinson's disease and 200 healthy controls) from >20 sites in the United States, Europe, and Australia. The study has since expanded to include subjects without evidence of dopaminergic deficit (SWEDD), a prodromal

cohort of participants with hyposmia or REM sleep behavior disorder, and a cohort of people with genetic mutations associated with a higher risk of developing Parkinson's disease. The study now runs at 33 clinical sites around the world. All subjects are comprehensively assessed at baseline and every three to six months thereafter. Subjects undergo clinical (motor, neuropsychiatric and cognitive) and imaging assessments and donate blood, urine, and cerebral spinal fluid (CSF). Data are collected by each site under uniformly-established protocols and data is stored and analyzed at designated core facilities. To date, this initiative has been very successful with high compliance with CSF collection and a 93% retention rate. Enrollment, as of April 2016, is 423 *de novo* Parkinson's disease patients, 196 healthy controls, 64 SWEDD subjects, 65 prodromal subjects and 245 subjects with genetic mutations. Recruitment for the genetic cohort is ongoing, with a goal of enrolling 600 subjects. This new cohort includes people with LRRK2, GBA and Synuclein (SNCA) mutations (31).

**Main inclusion criteria**:

**Parkinson disease (PD) Subjects:**

Inclusion:

Patients must have at least two of the following: resting tremor, bradykinesia, rigidity (must have either resting tremor or bradykinesia); OR either asymmetric resting tremor or asymmetric bradykinesia.

A diagnosis of Parkinson disease for two years or less at Screening.

Hoehn and Yahr stage I or II.

Confirmation from imaging core that screening DAT scan is consistent with Dopamine Transporter deficit. Assessment will be qualitative at baseline and quantitative at follow-up

Not expected to require PD medication with at least six months from Baseline.

Male or female age 30 years or older at time of PD diagnosis.

**Healthy Control (HC) Subjects:**

Inclusion:

Male or female age 30 years or older at Screening.

Ability to provide written informed consent.

Willing to comply with scheduled visits; women are not pregnant or lactating.

There are a total of 33 PPMI global clinical sites (http://www.ppmi-info.org/about-ppmi/ppmi-clinical-sites/)

**Main exclusion criteria**:

**Parkinson disease (PD) Subjects:**

Exclusion:

Currently taking levodopa, dopamine agonists, Monoamine oxidase B (MAO-B) inhibitors, amantadine or other PD medication.

Has taken levodopa, dopamine agonists, MAO-B inhibitors or amantadine within 60 days of Baseline.

Has taken levodopa or dopamine agonists prior to Baseline for more than a total of 60 days.

Received any of the following drugs that might interfere with DAT imaging: Neuroleptics, metoclopramide, alpha methyldopa, methylphenidate, reserpine, or amphetamine derivative, within 6 months of Screening.

Current treatment with anticoagulants (e.g., Coumadin, heparin) that might preclude safe completion of the lumbar puncture.

Condition that precludes the safe performance of routine lumbar puncture, such as prohibitive lumbar spinal disease, bleeding diathesis, or clinically significant coagulopathy or thrombocytopenia.

Use of investigational drugs or devices within 60 days prior to Baseline (dietary supplements taken

outside of a clinical trial are not exclusionary, e.g., coenzyme Q10).

**Healthy Control (HC) Subjects:**

Exclusion:

Current or active neurological disorder.

First degree relative with idiopathic PD (parent, sibling, child).

Montreal Cognitive Assessment (MoCA) score < 26.

Received any of the following drugs that might interfere with DAT imaging: neuroleptics, metoclopramide, alpha methyldopa, methylphenidate, reserpine, or amphetamine derivative, within six months of Screening.

Current treatment with anticoagulants (e.g., Coumadin, heparin) that might preclude safe completion of the lumbar puncture.

Condition that precludes the safe performance of routine lumbar puncture, such as prohibitive lumbar spinal disease, bleeding diathesis, or clinically significant coagulopathy or thrombocytopenia.

Use of investigational drugs or devices within 60 days prior to baseline (dietary supplements taken outside of a clinical trial are not exclusionary, e.g., coenzyme Q10).

**Subjects defined as SWEDD were identified *after* being recruited into the study. All inclusion criteria matched that of PD subjects with the exception that their scans showed no evidence of dopamine deficiency.**

**The primary study outcome is:**

The mean rates of change and the variability around the mean of clinical, imaging and biomic outcomes in early PD patients, and where appropriate the comparison of these rates between PD patient subsets and between PD and healthy subjects at study intervals from three months to 36 months. Specific examples of outcomes include MDS-UPDRS, DAT striatal uptake, and serum and CSF alpha-synuclein. PD patient subsets may be defined by baseline assessments, progression milestones and/or rate of clinical, imaging, or biomic change.

**The secondary outcomes are:**

Correlations between the rates of change in the mean of clinical, imaging and biomic outcomes in early PD patient subsets and between PD and healthy subjects at study intervals from three months to 36 months.

Prevalence of measures of clinical, imaging and biomic outcomes in early PD patients and healthy subjects at study intervals from baseline to 36 months.

To establish the predictive value of early clinical non-motor features, baseline imaging and biomic outcomes for future course of disease.

**Data Access**:

Data will be securely stored at central data coordinating facilities and will have all personally identifiable information removed before it is shared outside the study. All organizations responsible for data storage will observe the highest precautions to ensure data integrity and security. It is the goal of PPMI to enable timely access to the data by the PD research community.

**PPMI Statistical Methods:**

Changes from baseline to the one-year, two-year and three-year evaluations will be calculated and summarized descriptively. We will calculate 95% confidence intervals for the mean rate of change and between subject variability. For this purpose, the between subject variability will be estimated by fitting mixed models to all available data. Correlations will be calculated between the different measures, for example between change in total MDS-UPDRS and change in DAT uptake or alpha-synuclein levels.

770 References: (2) (31) (30)

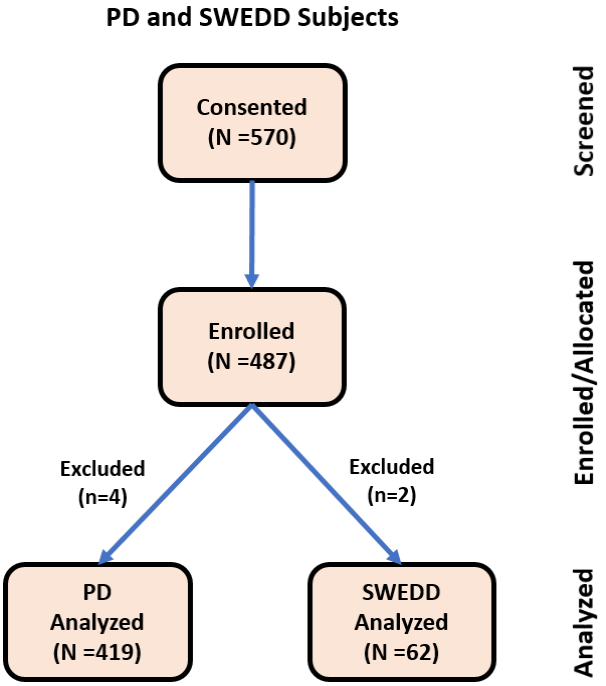



Figure A3 Schema for the Parkinson's Progression Markers Initiative (PPMI).

Of the 423 individuals in the PD cohort, four lacked baseline SWEDD status data and were thus excluded from the analysis, leaving a total of 419 individuals from the PD cohort from PPMI included in the analysis dataset. Of the 64 individuals in the SWEDD cohort, two lacked baseline SWEDD status, leaving a total of 62 from the SWEDD cohort from PPMI included in the analysis dataset. As such, patients with at least one observation were used in the analysis.

**Aligning target patient populations**

The conventional accepted diagnostic criteria for PD are according to the UK Parkinson's disease brain bank (UKPDBB) (6). Table A3 illustrates the features of diagnostic criteria in PPMI and PRECEPT relative to the UKPDBB criteria, and highlights the similarity across the data sources in terms of target population and relevance to proposed context-of-use for the biomarker in prospective trials. The proposed target population for use of the biomarker in prospective clinical trials is fully aligned with the target population in the data sources used in the analyses. Table A4 demonstrates that baseline demographic features are similar between SWEDD and DAT-deficient subjects in both PPMI and PRECEPT.

Technical aspects of the data acquisition and reconstruction of DAT SPECT images were matched between the SWEDDs and PD participants. In PPMI, much effort was devoted to the standardization of dopamine transporter SPECT for acquisition, reconstruction, visual assessment, and quantitation. Prior to scanning patients, each nuclear medicine site was physically visited by a technical setup team where the camera was assessed, the protocol for acquisition developed, and training provided for the local staff. All data were sent as raw projection files to the central core lab where 3D image reconstruction was performed in a consistent manner, with appropriate masking. Images were quality control-checked for adherence to the protocol and the quality and completeness of the data. For PPMI, all subjects (SWEDD and DAT-deficient) were aligned in terms of their imaging acquisition protocol including the time interval between injection and SPECT reading (4 hours in duration). In the PRECEPT trial, all imaging was done on a single research SPECT camera, and the data managed by the core lab research group, with appropriate masking.

799 Table A3 Features of diagnostic criteria in PPMI and PRECEPT relative to the UKPDBB criteria

| | United Kingdom Parkinson's Disease Brain Bank (UKPDBB) (6) | PPMI (final protocol, November 2012) | PRECEPT (22) |
|---|---|---|---|
| Key inclusion criteria | Bradykinesia AND at least one of the following:<br>- Muscular rigidity<br>- 4-6 Hz rest tremor<br>- Postural instability not caused by primary visual vestibular, cerebellar, or proprioceptive dysfunction | At least two of the following: resting tremor, bradykinesia, rigidity (must have either resting tremor or bradykinesia) OR either asymmetric resting tremor or asymmetric bradykinesia (e.g., diagnosis by single sign) | At least two of the cardinal signs of PD (resting tremor, bradykinesia, rigidity) |
| Other criteria | Supportive criteria (≥ 3 for diagnosis of definite PD):<br>- Unilateral onset<br>- Rest tremor<br>- Progressive disorder<br>- Persistent asymmetry most affecting the side of onset<br>- Excellent response to levodopa<br>- Severe L-dopa induced chorea<br>- L-dopa response ≥ 5 years<br>- Clinical course of ≥ 10 years | Other inclusion criteria:<br>- Diagnosis of PD for ≤ 2 years at Screening<br>- Hoehn and Yahr stage 1 or 2 at baseline<br>- Not expected to require PD medication within 6 months from baseline | Other inclusion criteria:<br>- Modified Hoehn and Yahr stage ≤ 2.5<br>- No current or imminent (in next 3 months) PD disability requiring dopaminergic therapy |
| Comments | - The validity of UKPDBB criteria is based on confirmation of clinical diagnosis by post-mortem exam<br>- Criteria are applied to well-established PD rather than early PD. | It is rare to have asymmetric rest tremor without bradykinesia or asymmetric bradykinesia without some increased muscle tone; therefore, the diagnosis based on a single sign represents an unusual situation. | |

800 Table A4 Baseline characteristics are similar between SWEDD and DAT-deficient subjects in
801 PRECEPT and PPMI

| Baseline | PPMI | | PRECEPT | |
|---|---|---|---|---|
| DAT imaging status | DAT Deficit | SWEDD | DAT Deficit | SWEDD |
| Sample size | 418 | 63 | 165 | 26 |
| Sex, % | Female (35), Male (65) | Female (38), Male (62) | Female (33), Male (67) | Female (38), Male (62) |
| Age in year, median (range) | 62 (33, 84) | 63 (38, 78) | 61 (31, 82) | 62 (32, 84) |

| | | | | |
|---|---|---|---|---|
| Harmonized motor scores, median (range) | 20 (4, 51) | 13 (2, 42) | 20 (7.1, 52) | 14 (5.3, 28) |

**Harmonization of UPDRS and MDS-UPDRS Motor Scores**

While MDS-UPDRS was used in PDMI trial, UPDRS was used in PRECEPT trial. To combine the data from the two trials, UPDRS scores were converted to MDS-UPDRS scores. The CPP Team will focus on part III, which represents the motor score. Analytic approaches will follow the recommendations of (16) (Table A5).

Table A5 Conversion formulas for testing MDS-UPDRS scores derived from UPDRS scores for each part of the MDS-UPDRS, calibrated for different Hoehn and Yahr groupings.

To convert a UPDRS score to a comparable MDS-UPDRS score, the UPDRS Part score is multiplied by the weighting factor and the product is summed with the intercept, with the final value rounded to the nearest integer. Weighting factors and intercepts have been truncated to a single decimal for ease of use. Gray portions (Parts II and III) provided significant calibration formulas for transformation of UPDRS scores to MDS-UPDRS scores. White portions (Parts I and IV) failed to provide significant calibration formulas. Figure 1 from (16).

| H & Y Stage | MDS-UPDRS Part I | MDS-UPDRS Part II | MDS-UPDRS Part III | MDS-UPDRS Part IV |
|---|---|---|---|---|
| I/II | (UPDRS Part I x 2.5) + 4.7 | (UPDRS Part II x 1.1) + 0.2 | (UPDRS Part III x 1.2) + 2.3 | (UPDRS Part IV x 1.0) - 0.3 |
| III | (UPDRS Part I x 2.0) + 7.7 | (UPDRS Part II x 1.0) + 1.5 | (UPDRS Part III x 1.2) + 1.0 | (UPDRS Part IV x 1.0) - 0.3 |
| IV/V | (UPDRS Part I x 1.6) + 10.8 | (UPDRS Part II x 1.0) + 4.7 | (UPDRS Part III x 1.1) + 7.5 | (UPDRS Part IV x 1.0) + 0.8 |

- Concordance between observed and UPDRS-derived MDS-UPDRS scores and based on Lin's concordance coefficient (LCC) for continuous level data
- LCC=0.93 for MDS-UPDRS Part II and 0.97 for Part III

**Imaging methodology**

**123I-Ioflupane Usage: Patient Requirements and Contraindications**

Contraindications for Use

The context-of-use specifies that reductions of DAT as assessed by SPECT neuroimaging will be utilized as an adjunct to clinical assessments for the purposes of enriching the patient population with subjects who have increased likelihood of having idiopathic PD. The subjects will have an objectively confirmed motor impairment with alternative identifiable causes of motor impairment appropriately excluded through clinical means prior to the use of DAT neuroimaging.

The following are contraindications to SPECT imaging:

- Pregnancy.
- A known hypersensitivity to the active substance or to any of its excipients. An iodine allergy is, however, not a contraindication to receiving this tracer.
- Inability to cooperate with SPECT or SPECT/CT brain imaging.
- Not of adult age. 123I-ioflupane is not indicated for use in children, as its safety and efficacy have not been established in pediatric patients.

- Breastfeeding. This is a relative contraindication, as it is not known if 123I-ioflupane is excreted into human milk. For caution, if the test remains indicated, nursing women may consider pumping and discarding breast milk for at least 1 day and perhaps up to 6 days after tracer administration (32) (9).

**Concomitant medications**

Certain medications have been identified with the potential to interfere pharmacologically with binding of 123I-Ioflupane to its ligand in vivo. Table 1, as published in the EU Nuclear Medicine review (9), lists these medications. Such medication use is not recommended for patients undergoing SPECT imaging where quantitative measurements will be desired. Importantly, however, such drugs are unlikely to impact interpretation of visual reads according to the context-of-use for the CPP imaging biomarker application under review for qualification. The combination of the large reduction in DAT imaging signal at time of diagnosis with the fact that such drugs would be unlikely to impact either the signal to noise ratio in caudate vs. putamen or the symmetry of uptake in ipsilateral vs contralateral putamen makes it unlikely for these drugs to interfere with interpretation of the visual reads. Furthermore, the patient population is aimed at early motor PD at a time when subjects are not being treated with multiple medications. Despite this, in an ongoing clinical study focused on biomarker investigations (S4 study, systemic synuclein sampling study, https://www.michaeljfox.org/page.html?s4), subjects taking such medications are advised to withdraw medication for six hours prior to the scan. This is a reasonable recommendation for sponsors proposing to use DAT imaging for subject selection as per the proposed context-of-use.

The following additional medications should not interfere with visual interpretation:

- Selective serotonin reuptake inhibitors may increase binding to DAT somewhat but should not interfere with visual interpretation (33).
- Cholinesterase inhibitors and neuroleptics probably do not interfere significantly with 123I-ioflupane binding to DAT (33).
- Anti-Parkinsonian drugs (e.g., L-dihydroxyphenylalanine, dopamine agonists, monoamine oxidase B inhibitors, N-methyl-D-aspartate receptor blockers, amantadine, and catechol-O-methyltransferase inhibitors in standard dosages) do not interfere with 123I-ioflupane binding to DAT to any significant degree (33) (34).

An extensive overview of drug influences on DAT SPECT can be found in an article by Booij and Kemp (33).

**Equipment specifications and quality control**

A multi-detector SPECT γ-camera is advised for image acquisition. A single-detector camera may provide less than optimal resolution, and is not recommended (35). Low-energy high-resolution or low-energy ultra -high-resolution parallel-hole collimators are most commonly used for brain imaging and provide acceptable images of diagnostic quality.

For extrinsic uniformity calibrations, the use of a 123I flood source may be more rigorous than 99mTc or 57Co flood sources, and should be performed on a daily basis. Uniformity of response to a uniform flux of radiation from a 123I point source should be measured intrinsically every quarter (36). Other routine Quality Control procedures recommended for 123I-ioflupane are listed below (36) (37):

- Transaxial Uniformity (quarterly, using a uniform phantom)

- Center of Rotation Alignment (quarterly)
- Sensitivity Calibration (quarterly)

**Site qualification**

Site qualification steps would include:

- Individual site visits and image protocol set-up with verification that equipment meets the specifications described in Equipment Specifications and Quality Control, optimization of camera protocols, and standardization of centers' processing methods.
- A phantom scan typically performed during set up by the tracer manufacturer.
- Ongoing core imaging lab assessment of images as they are obtained via rapid quality control check of the imaging data submitted to the core lab and feedback to the imaging center.
- Discussions with site staff to ensure a common understanding of requirements.

**Protocol / Image acquisition**

Timing

SPECT should be started when the ratio of striatal to occipital 123I-ioflupane binding is stable, between 3 and 6 hours after injection of the radiotracer (38) (39). It is recommended that each center use a fixed interval that is consistent across the study and to other studies against which results may be compared between tracer injection and image acquisition to optimize reproducibility and to limit inter- and intra-subject variability.

Positioning

Patients should be encouraged to void before scanning to avoid disturbance during image acquisition; should be positioned supine, with head centered and as straight as possible; and should be instructed to remain still during the acquisition. An off-the-table headrest is essential to minimize the radius of the camera orbit and a flexible head restraint such as a strip of tape across the chin or forehead may be used to minimize movement.

Although proper alignment with no head tilt would be preferable, patient comfort is more important than the actual orientation of the head, as long as the striatum (the caudate nucleus and putamen) and occipital cortex are in the field of view. If necessary, images can be reoriented after the acquisition.

Patients who prefer to lie with the knees slightly bent may need supporting cushions. Binding the shoulders (e.g., with a sheet) may also help to prevent movement as well as to reduce the orbital radius of the camera heads.

If a patient is not able to remain still, and if the referring physician and patient's legal representative agree, sedation with short-acting benzodiazepines can be used (and will not affect scan quality). If sedation is used and the patient traveled to the clinic by car, there should be an accompanying person to drive the patient home (32) (9).

Image Acquisition

Ideally the field of view should include the entire brain although if, for example, the exclusion of the cerebellum allows a smaller rotational radius, this is not essential. The typical radius of a circular

acquisition is 11–15 cm and the mean radius of an elliptical acquisition should also fall within this range but not exceed 18 cm.

The photopeak should be set to 159 keV ± 10%. Additional energy windows may be used for scatter correction purposes.

A 128 × 128 matrix is recommended. Experimental studies with a striatal phantom suggest that optimal images are obtained when the selected matrix size and zoom factors give a pixel size of 3.5–4.5 mm. Slices should be 1 pixel thick.

Step-and-shoot mode with angle increments of 3° is recommended. Full 360° coverage of the head is required (i.e., 180° for each head of a dual-head camera). The number of seconds per position depends on the sensitivity of the system, but usually 30–40 s are required.

A minimum of 1.5 million total counts should be collected for optimal images for parallel-hole collimator systems, and the acquisition time will vary according to the camera specifications. The acquisitition time is often is the range of 30–45 minutes (32) (9). There are no data that support a rationale for variable SPECT acquisition mode parameters, specifically the acquisition time depending on subject weight and or amount of injected 123I (36).

For SPECT/CT systems, the CT should be configured to acquire a low-dose non-diagnostic quality scan. When used for attenuation correction only, the CT can be performed with 5-10 mAs, and when used for anatomic localization, the CT can be performed with 30-60 mAs. Other recommended CT acquisition parameters include 110-130 kVp and pitch set to 0.8-1.5 (36). Scanner-specific acquisition parameters are found in the DaTscan™ Protocol Manual published by GE Healthcare (32).

**Image processing**

A first step in image processing should involve review of projection data in cine mode and sinograms for an initial determination of scan quality, patient motion, and artifacts.

Images are then reconstructed preferably using iterative reconstruction, but filtered back-projection may be used. The reconstructed pixel size should be 3.5 to 4.5 mm with slices 1 pixel thick (36).

In normal circumstances the striatum should be the brain region with highest intensity in the field-of-view and this governs the display scaling. Axial reconstruction limits should be adjusted such that any uptake present in the salivary glands, which could swamp the striatal signal, is outside the field-of-view.

A low-pass filter (e.g., Butterworth) is recommended. Other types of filters can introduce artifacts, may affect the observed or calculated striatal binding ratio, and should be used with caution. The filter should preserve the linearity of the count rate response. Filtering includes either a 2-dimensional pre-filtering of the projection data or a 3-dimensional post-filtering of the reconstructed data.

While attenuation correction is recommended, it can introduce its own artifacts and is not essential. An attenuation map can be measured from a simultaneously or sequentially acquired transmission or CT scan, or can be calculated, as with a correction matrix according to Chang (40). The broad-beam attenuation coefficient is typically assumed to be 0.11 cm−1. Some variance may occur with fan-beam collimators. Accuracy may be verified with an appropriate 123I phantom (41).

Images are reformatted into slices in three planes (axial, coronal, and sagittal). Correct reorientation makes visual interpretation easier and is crucial when semi-quantification is used. Transverse slices should be parallel to a standard and reproducible anatomic orientation, such as the anterior commissure–posterior commissure line as used for brain MRI. This can be approximated by orientating

the brain such that the inferior surface of the frontal lobe is level with the inferior surface of the occipital lobe. The canthomeatal plane, as routinely used for CT, is also acceptable. Activity in the striatum and the parotid glands, and the contours of the brain and the head, can usually be seen and can be used to assist realignment. A simultaneously acquired CT scan may allow more precise realignment of the head.

**Image interpretation**

Image Quality

Prior to attempting to read and interpret the image, the reader should verify the quality of the acquired images. The alignment of the head should be checked, since misalignment may create artificial asymmetry and may lead to misinterpretation of the scan. If the maximal intensities of striatal regions occur in different transverse planes, this may be indicative of uncorrected head tilt.

Visual Interpretation

In visual interpretation, the striatal shape, extent, symmetry, and intensity differentiate normal from abnormal. The normal striata on trans-axial images should look crescent- or comma-shaped and should have symmetric well-delineated borders. Abnormal striata will have reduced intensity on one or both sides, often shrinking to a circular or oval shape.

The level of striatal activity should be compared with the background activity. Both orthogonal slices and multiple-intensity-projection images can be used. The head of the caudate and the putamen should have high contrast to the background in all scales and for patients of all ages. Image interpretation is based on evaluation of the entire axial image as opposed to individual slices.

Some decrease in striatal binding, in both the caudate and putamen, occurs with normal aging ( ≃5% 7% per decade). This decrease is small in comparison to the decreases caused by disease and normally should not interfere with interpretation (42).

The left and right striata should be rather symmetric in the healthy state; mild asymmetry may occur in normal subjects. Often, disease first becomes visible in the putamen contralateral to the neurologic signs (43).

Image interpretation should be performed on the computer screen rather than a hard copy because the image may need to be adjusted for alignment, scaling, and color. Scans should be analyzed in both gray scale and color. Readers are recommended to select one color scale with which to become familiar, consistent, and well-versed.

The recommended procedure for visual read of 123I-ioflupane images is based upon three distinctive steps in the evaluation carried out by blinded readers: 1) verify caudate nucleus neuroanatomically; 2) assess left vs right signal in caudate and putamen and 3) systematically evaluate and define binary classification (yes/no) evidence of presynaptic dopaminergic deficit consistent with Parkinsonism. This information is used to define SWEDD status.

**Training of the blinded readers**

Reader Qualifications

The qualified reader is a board certified Nuclear Medicine Physician with subspecialty expertise in neuroimaging. The process of the visual assessment will be reviewed in detail with the readers. All readers participating in the blind read will be familiar with the clinically available 123Iodine-Ioflupane

SPECT scan interpretation as part of their standard routine clinical nuclear medicine practice. Training will be built upon concepts learned in the assessment of these images.

Training Process

Readers will be trained in the visual assessment of dopamine transporter uptake in normative and Parkinson's disease patient populations by a qualified reader. The first part of the training session includes a review of the appropriate study procedures, a review of the image display software (i.e., how to ensure proper windowing of the image and adjust head rotation, if needed), a review of the electronic Case Report Forms (eCRF) including the plausibility rules, and a review of preselected training cases.

As further background training for readers, GE Healthcare has developed a set of training videos that can be accessed on-line at http://us.datscan.com/elearning/.

Reader performance verification

All readers will be tested with 20 review cases that they will perform at their designated workstations along with their proctors present and will be expected to correctly assess at least 80% of the training images in order to proceed on to the official read. If readers score below 80%, additional training would be administered and the reader will be tested with a different set of training images. Again, the reader must score 80% or higher. If the reader scores below 80% on the second set of training images they will be excluded from the read and not replaced.

All readers are expected to achieve 100% accuracy during the initial training and testing session. Therefore, a secondary list of additional training images will be used if needed to ensure accuracy of readers prior to evaluation of test images for the study.

**Documentation/Reporting**

History

State whether the patient used interfering drugs, and if so, which drugs. If sedation had to be performed, describe the route, dosage, and timing in relation to the scan.

Technique

State the time that elapsed between tracer injection and acquisition. State the injected radiopharmaceutical dose. State what criteria are used for the report interpretation (e.g., visual assessment, semi-quantitative analysis, or comparison to reference database).