Independent Review Charter: SPECT and Tau PET

Document Version	Final v3.0; 28-Jan-2022
Sponsor for Main Study	Michael J. Fox Foundation
Main Study	
Protocol Identifier	002
Protocol Version	Version 1.2; 01-Feb-2021
Protocol Description	The Parkinson's Progression Markers Initiative (PPMI) Clinical - Establishing a Deeply Phenotyped PD Cohort
Sponsor for Companion Study	Institute for Neurodegenerative Disorders
Companion Study	
Protocol Identifier	004
Protocol Version	Version 2; 19-Jun-2020
Protocol Description	Early Longitudinal Imaging in Parkinson's Progression Markers Initiative Using [^{18}F] AV-133 and DaTscan™ (PPMI Early Imaging 2.0)
Sponsor for Tau PET Study	Michael J. Fox Foundation
Companion Study	
Protocol Identifier	008
Protocol Version	Version 1.0; 26-Apr-2021
Protocol Description	Assessment of Brain Tau Burden in Participants with Parkinson's Disease in the PPMI Study (PPMI Tau PET Imaging)

Document Approval

Sponsor Signature Page

The signatures below, in addition to all other designated signatures, indicate that this document is accepted and approved for implementation.

Document Title	Independent Review Charter: SPECT and Tau PET
Document Version	Final v3.0; 28-Jan-2022
Protocol Identifier	002 and 004

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Document Title	Independent Review Charter: SPECT and Tau PET
Document Version	Final v3.0; 28-Jan-2022
Protocol Identifier	002, 004, and 008

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Table of Contents

1 Executive Summary

The 002, 004, and 008 clinical trials are sponsored by Michael J. Fox Foundation (MJFF) in collaboration with the Institute for Neurodegenerative Disorders (IND). MJFF and IND have designated Invicro as the medical imaging operations organization. Invicro will conduct central analyses (Independent Review) of neuroradiological imaging acquired by the investigational neuroimaging centers (sites) for the clinical trial protocol (protocol) 002, 004 and 008. Invicro will transfer the Independent Review results to MJFF or designee for statistical analysis.

1.1 Statement of Compliance

The Independent Review will be conducted in accordance with International Council for Harmonization E6(R2) guidelines on Good Clinical Practice (GCP),[1] the United States Food and Drug Administration (FDA) 2018 guidance on clinical trial imaging endpoint process standards,[2] the FDA guidance on Computerized Systems,[3] and the United States Code of Federal Regulations Title 21 Part 11 (21CFR §11).[4]

Any amendment to the Single-Photon Emission Computed Tomography and Tau Positron Emission Tomography Independent Review Charter (SPECT and Tau PET Charter) must be reviewed and approved by IND on behalf of MJFF.

1.2 Purpose and Scope of the Independent Review Charters

The purpose of the SPECT and Tau PET Charter is to outline the trial-specific processes intended to control for potential bias or variability during image acquisition, submission to Invicro, management, interpretation, and transfer to MJFF or designee. The SPECT and Tau PET Charter should be considered supplementary to the protocols 002, 004, and 008.

The scope of the SPECT and Tau PET Charter outlines the activities necessary to conduct the Independent Review, specific to SPECT and Tau PET imaging. This encompasses trial- and modality-specific image acquisition parameters; site training and engagement (including approval of phantom scans for scanner qualification); management of image data submitted by the sites; the Independent Review paradigm; software configuration and integration; compliant documentation; and, the management and delivery of Independent Review results data. For details on magnetic resonance imaging (MRI), refer to the approved Independent Review Charter: MRI for study 002. For details on [^{18}F] AV-133 PET imaging, refer to the approved Quantitative Analysis Methodology: [^{18}F] AV-133 for the companion study, 004.

1.3 Trial Overview and Function of Imaging

1.3.1 Trial Design

1.3.1.1 Main Study (002)

The main study (protocol 002) is a longitudinal, observational, multi-center natural history study to assess progression of clinical features, digital outcomes, and imaging, biologic and genetic markers

of Parkinson's Disease (PD) progression in study participants with manifest PD, prodromal PD, and healthy controls. The trial will identify markers of disease progression for use in clinical trials to reduce progression of PD disability.

The primary objectives of this study are to:

a. Establish standardized protocols for acquisition, transfer and analysis of clinical, digital, imaging, biologic and genetic data that can be used by the PD research community. This protocol will build on the existing PPMI infrastructure.
b. Develop a comprehensive and uniformly acquired clinical, digital and imaging dataset and repository of biological and genetic samples that would be available to the PD research community to test hypotheses of the underlying molecular pathobiology of PD, enable modeling of PD progression to identify clinical and/or data driven PD progression subsets, and inform studies testing PD therapeutics (for examples, clinical trials targeting synuclein, LRRK2 (Leucine-rich repeat kinase 2), GBA (glucocerebrosidase gene) as well as other targets)
c. Use clinical and biological data to estimate the mean rates of change and the variability around the mean of clinical, digital, imaging, biological and genetic outcomes in study participants with PD diagnosis (including patients with a LRRK2, GBA, SNCA [Alpha-synuclein]) or rare genetic mutations (such as Parkin or Pink1) and individuals with prodromal Parkinson disease (including individuals with RBD [REM Sleep Behavior Disorder]), olfactory loss, LRRK2, GBA, SNCA or rare genetic variants (such as Parkin or Pink1) and/or other risk factors for PD with and without DAT (dopamine transporter) deficit and in healthy participants.
d. Confirm existing and identify novel clinical, digital, imaging, biologic and genetic PD progression markers to identify quantitative individual measures or combinations of measures that demonstrate optimum interval change in study participants with PD diagnosis (including patients with a LRRK2, GBA, SNCA or rare genetic variants [such as Parkin or Pink1]) and individuals with prodromal Parkinson disease (including individuals with RBD, olfactory loss, a LRRK2, GBA, SNCA or rare genetic variants [such as Parkin or Pink1]) and/or other risk factors for PD with and without DAT deficit in comparison to healthy controls or in subsets of study participants with PD diagnosis or prodromal PD defined by baseline assessments, progression milestones and/or rate of clinical, digital, imaging, biologic and genetic change, or other measures.
e. Evaluate the probability of phenoconversion to PD for individuals with prodromal PD enrolled in the prodromal cohorts (including individuals with RBD, olfactory loss, a LRRK2, GBA, SNCA or rare genetic variants (such as Parkin or Pink1) and/ or other risk factors for PD with and without DAT deficit).

The secondary objectives of this study are:

a. Conduct preliminary clinical, digital, imaging, biologic and genetic markers verification studies on promising biological markers in study subsets and/or using stored collected samples.
b. Compare biomarker signatures for study participants with PD diagnosis without known genetic mutation to those with known genetic mutation (including LRRK2, GBA, SNCA or rare genetic variants [such as Parkin or Pink1]).
c. Compare biomarker signatures in study participants with PD diagnosis to individuals with prodromal PD enrolled in the prodromal cohorts (including individuals with RBD, olfactory loss,

LRRK2, GBA, SNCA or rare genetic variants (such as Parkin or Pink1) and/or other risk factors for PD with and without DAT deficit).

d. Compare biomarker signature between prodromal PD subsets including individuals with RBD, olfactory loss, LRRK2, GBA, SNCA or rare genetic variants (such as Parkin or Pink1) and/or other risk factors for PD with and without DAT deficit.
e. Develop and test risk paradigms to establish the sequence of early prodromal events (clinical, imaging, biologic changes) in individuals with prodromal PD enrolled in the prodromal cohorts (including individuals with RBD, olfactory loss, LRRK2, GBA, SNCA or rare genetic variants (such as Parkin or Pink1) and/or other risk factors for PD with and without DAT deficit) including testing early signal of risk in the associated PPMI Online and PPMI Remote studies.

The PPMI Clinical study will include up to 4,500 participants at up to 50-55 international clinical sites. Participants will be in one of the following cohorts:

- Current PPMI 001 participants will be divided into the following cohorts:
    - Healthy Control
    - PD
    - Prodromal
- Newly enrolled PPMI Clinical participants will be divided into the following cohorts:
    - Healthy Control
    - PD
    - Prodromal

For the main study (002), participants will receive up to 6 DAT SPECT scans, depending on the cohort designation. DAT SPECT scans will be used to evaluate disease progression through imaging biomarkers. DAT SPECT scans will also be used to evaluate eligibility. For full details on SPECT imaging see Section 2.2. For details on MRI imaging performed in the main study, refer to the approved Independent Review Charter: MRI for study 002.

1.3.1.2 Companion study (004)

The companion study (protocol 004) is a longitudinal, multi-center study to assess progression of DAT SPECT and [^{18}F] AV-133 imaging in PD participants for at least 18 months. Approximately 50 PD participants from the main study will be recruited from up to 5 clinical sites. All participants will be comprehensively assessed at baseline and every 6 months thereafter.

For the companion study (004), participants will have up to 3 DAT SPECT scans, depending on if the participant is transitioning from PPMI 001 to PPMI Clinical or newly enrolled in PPMI Clinical. DAT SPECT scans will be used to evaluate the predictive value of early imaging. For full details on SPECT imaging see Section 2.2. For details on [^{18}F] AV-133 PET, including the imaging schedule and analysis, refer to the approved Quantitative Analysis Methodology: [^{18}F] AV-133 for study 004.

1.3.1.3 Tau PET study (008)

The Tau PET study (protocol 008) is an imaging study to test whether PET with [^{18}F]PI-2620 can visualize in vivo brain tau deposition in participants with PD. Approximately 35 participants enrolled in

the main study will be recruited, including approximately 10 individuals with LRRK2 mutations (manifest or non-manifest), 20 sporadic PD individuals (across a range of disease duration from newly diagnosed to longstanding PD) and 5 healthy controls.

Tau PET imaging will be used to assess the regional brain uptake of [^{18}F]PI-2620 targeting brain tau assessed with PET imaging. The primary objective of this study is to test whether PET with [^{18}F]PI-2620 can visualize in vivo brain tau deposition in participants with PD, and the secondary objective of this study is to evaluate tau deposition in PD LRRK2 mutation carriers, given recent data that tau pathology may be present in those individuals. For the Tau PET study (008), participants will have one Tau PET scan with radiotracer [^{18}F]PI-2620. For full details on Tau PET imaging see Section 2.2.

1.3.2 Role of SPECT Imaging in the Trial

1.3.2.1 Eligibility for the main study (002)

Eligibility for participants newly enrolled in PPMI Clinical will be determined through qualitative and quantitative assessments of screening DAT SPECT images. Current PPMI 001 participants will not require imaging for eligibility.

Invicro will perform qualitative assessment of images for participants in the Healthy Control and PD cohorts. Healthy Control participants must have normal levels of striatal dopamine transporter as determined by the Independent Review. Participants with PD must have evidence of abnormal (ie, decreased) levels of striatal dopamine transporter as determined by the Independent Review.

Invicro will perform quantitative analysis of images for participants in the Healthy Control, PD, and Prodromal cohorts for determining participant eligibility using pre-specified cut-offs.

Results of the qualitative and quantitative assessments will be transferred to Blackfynn, Inc (Blackfynn) who will determine eligibility through a pre-determined algorithm.

For protocol 002, previously-acquired SPECT scans from a prior clinical investigation may also be used to determine study eligibility. See Section 0 for additional details on the use of previously-acquired scans.

1.3.2.2 Longitudinal changes in imaging Biomarkers for the Companion Study (004)

SPECT imaging will support the study outcomes for longitudinal changes in quantitative DAT SPECT imaging and striatal binding ratio (SBR). Invicro will perform the quantitative analyses in support of the assessment in longitudinal changes in imaging biomarkers.

1.3.3 Role of Tau PET Imaging in the Trial for the Tau PET Study (008)

In the Tau PET study (008), Tau PET imaging will be used to support the study outcomes of evaluating the regional brain binding of [^{18}F]PI-2620 in support of the primary and secondary objectives of the trial. Invicro will perform the quantitative analyses in support of the regional assessment of Tau PET imaging.

Note: Baseline MRIs from the main study (002) will be integrated in the Tau PET image analysis pipeline for registration and normalization by Invicro. For full details on MRI, see the approved Independent Review Charter: MRI.

1.3.4 Function of the Independent Review

The Independent Review performed at Invicro will function as a centralized, objective, and systematic analysis of imaging. Invicro will analyze SPECT and Tau PET scans, with the Independent Review divided into the following components:

- **SPECT Visual Assessment** (Section 4) — A qualitative review performed by independent Reviewers to determine an overall assessment of normal or abnormal levels of dopamine transporter. Agreement of a positive rating from 2 of 3 Reviewers will provide overall interpretation of the scan.
- **SPECT Quantitative Analysis** (Section 5) — To quantitatively assess binding of the radiotracer by measuring the SBR.
- **Threshold Analysis for Eligibility** (Section 6) — To determine eligibility based on threshold analysis of SBRs.
- **Tau PET Quantitative Analysis** (Section 7) — To quantitatively assess regional binding of [^{18}F]PI-2620 by measuring the standardized uptake values (SUVs) and standardized uptake value ratios (SUVRs).

Note: Invicro will not be performing the eligibility determination based on threshold analysis.

1.4 Roles and Responsibilities

1.4.1 MJFF

MJFF and designated contract research organization(s) are responsible for the overall conduct of the trial, including protocol design, regulatory compliance, clinical site designation, imaging center designation, all contracting and payment, regulatory and ethics committee submissions, participant recruitment, clinical site monitoring, safety monitoring and reporting, clinical data management, statistical analysis, and regulatory reporting. MJFF and designee are also responsible for statistical management of results data.

1.4.2 Invicro

Invicro will be responsible for facilitating the Independent Review processes and managing the image data workflow for SPECT (Figure 1) and Tau PET (Figure 2) imaging.

Figure 1: Overview of the SPECT Image Data Workflow

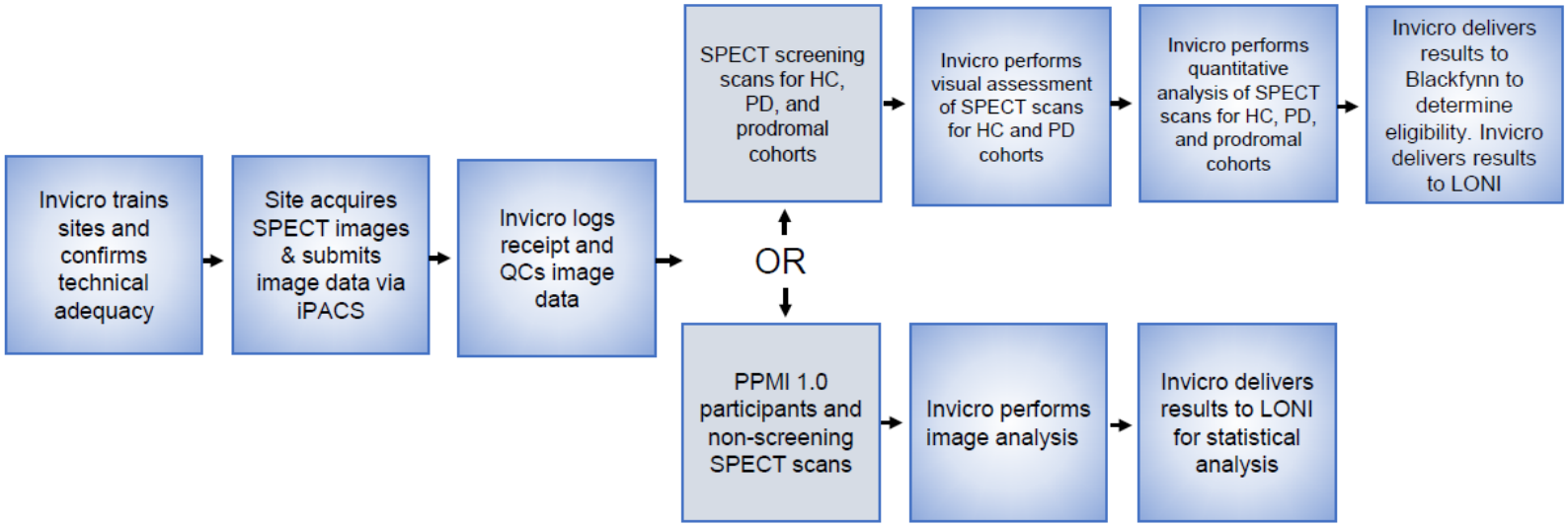
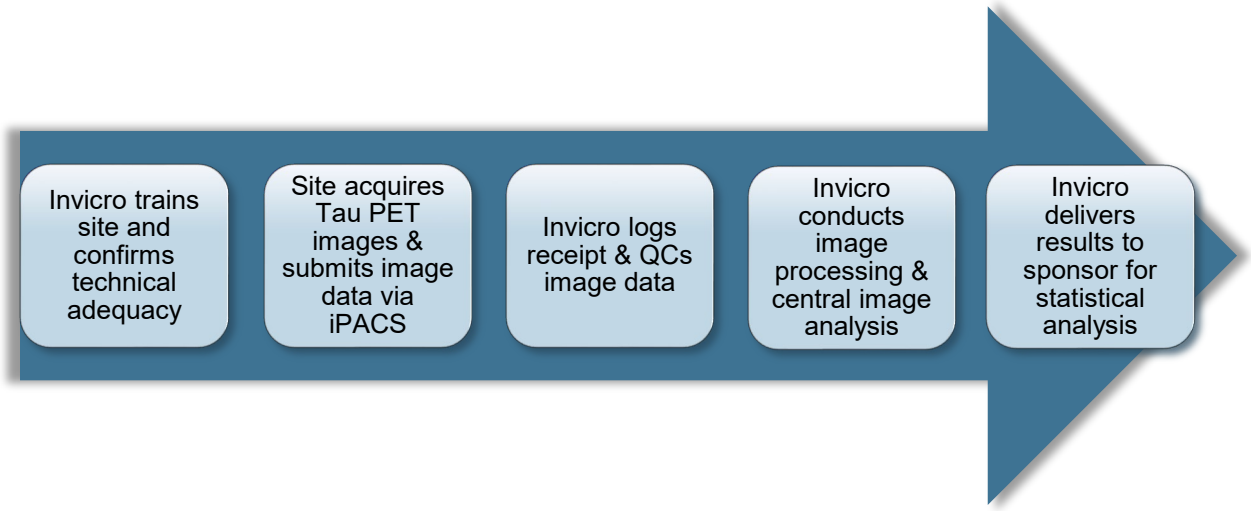


Figure 2: Overview of the Tau PET Image Data Workflow



Invicro will work with MJFF and/or designee as appropriate to complete the following specific activities:

- Design and planning
  - Image acquisition parameters specific to modality and the protocol
  - Management of image data, review processes, quantitative image analysis, and data delivery schedule

  - Independent Review paradigm and operations; SPECT and Tau PET Charter development

- Site interaction
  - Distribution of the SPECT Technical Operations Manual (TOM) and Tau PET TOM
  - Technical site set-up for SPECT and Tau PET acquisition
  - Training sites on acquisition parameters and submission processes
  - Ongoing site communication regarding image submission and the resolution of any queries related to incomplete or inaccurate submissions
- Image data management
  - Image quality control (QC) for adequate images analysis
- Coordination and conduct of Independent Review
  - Recommendation of expert Reviewers who will perform the DATSPECT Visual Assessment, contracting with those candidates
  - Configuration of the Independent Review database and the electronic case report form (eCRF) that captures assessment results
  - Integration of Baseline MRIs from the main study into the Tau PET imaging analysis pipeline
  - Pre-processing and quantitative analysis of DATSPECT and Tau PET performed by an Image Processing Specialist (IPS)
  - Post-review quality checks
  - Delivery of Independent Review results database for DAT SPECT Eligibility to Blackfynn and Laboratory of Neuro Imaging (LONI)
  - Delivery of Independent Review results database for DAT SPECT to LONI
  - Delivery of Independent Review results database for Tau PET to LONI
  - Preparation of the final study reports

1.4.3 Independent Reviewers for Visual Assessments

Reviewers are physicians with expert knowledge in the trial-specific disease indication(s) and with experience in clinical trial review. Reviewers will be certified by a recognized national or international medical accrediting agency (eg, American Board of Radiology).

Before evaluating any trial images, the Reviewers must complete training specific to the Independent Review with re-training during the trial as required.

1.4.4 Investigational Sites

Site personnel, including those who acquire, archive, and submit images are responsible for the following activities:

- Undergo training from Invicro on trial-specific imaging standards and image data submission
- Acquire images from each enrolled participant according to the trial-specific standards and the Schedule of Assessments outlined in the protocol
- Use the same scanner and repeat the same acquisition techniques throughout the duration of the participant's participation

The site investigator is responsible for image acquisition quality oversight, the prompt submission of image data to Invicro, and the timely response to queries generated by Invicro.

2 Site Image Acquisition and Submission to Invicro

2.1 Technical Operations Manuals and Acquisition Parameters

Invicro will prepare and distribute modality-specific TOMs for SPECT scans and Tau PET scans to the participating sites. The TOMs communicate the trial-specific process standards and modality-specific details designed to control for variability during image acquisition. The TOMs include the following content:

- Complete instructions on trial-specific image acquisition processes with required and recommended technical parameters
- Equipment qualification
- Technical details of equipment operation and image acquisition, including participant preparation, positioning, and comfort measures
- Trial-specific procedures regarding the standardization of drugs used for imaging (ie, preparative drugs, contrast agents, and/or radiopharmaceutical agents)
- Ordering procedures for radiopharmaceutical agents
- QC performed by the site personnel
- Site training and qualification process
- Schedule of imaging assessments
- Instructions for submitting images and required documentation to Invicro, including the software platform
- Image archiving by the site
- Query generation and resolution procedures for missing or inadequate image data
- Invicro study team contact information

Full details on all these topics may be referenced in the SPECT TOM and Tau PET TOM.

2.2 Scheduled Imaging

Sites will be instructed to acquire SPECT and Tau PET imaging according to the Schedule of Assessments (Table 1, Table 2, and

Table 3), as stipulated by the protocols, and according to the parameters in the TOMs. Sites will submit these images to Invicro for inclusion in the Independent Review and quantitative analysis.

^{123}I-DaTscan™ will be used as the radiotracer for DAT SPECT imaging. Select sites may be approved for use of ^{99m}Tc-TRODAT-1 due to limited availability of ^{123}I-DaTscan™.

Table 1: Schedule of SPECT Assessments for Main Study (Protocol 002)

Scan	Healthy Control [a,b]	PD [a,b]	Prodromal [a,b]
Screening Scan [c,d]	X	X	X
Visit 04 (12 months)[e]	-	X	X
Visit 06 (24 months)[e]	-	X	X
Visit 10 (48 months)[e]	-	X	X
Premature Withdrawal (PW)[f]	-	X	X
Diagnostic Visit [g]	-	-	X

[a] Cohorts include participants newly enrolled in PPMI Clinical and participants transitioning from PPMI 001. Newly enrolled participants in PPMI Clinical will start with the Screening Visit. Participants transitioning from PPMI 001 to PPMI Clinical will not have a screening DAT SPECT scan, and these participants will start at the equivalent visit in PPMI Clinical from the PPMI 001 schedule.

[b] Participants may not have the following drugs within 6 months of the Screening Visit: dopamine receptor blockers (neuroleptics), metoclopramide and reserpine.

[c] A previously-acquired screening DAT SPECT image may be used if the scan was acquired within 6 months of the study scheduled DAT SPECT Screening visit, and meets criteria of the Imaging Core Lab to accept the scan for use.

[d] DAT SPECT screening imaging appointments will occur within 60 days prior to Day 1.

[e] DAT SPECT imaging will occur within ±45 days either side of the Target Visit Date.

[f] If a participant withdraws from the study and agrees to attend one more visit, the next scheduled annual visit should be completed. DAT SPECT imaging will be performed if not done in the previous 6 months. The annual visits are nested within the “Other SPECT Timepoint” selection option when submitting the data to Invicro in iPACS.

[g] If the next planned visit is an annual study visit, this diagnostic visit will take the place of the next annual visit. The participant will undergo all assessments scheduled for that annual visit. If not already scheduled as part of that visit, or not completed in last 6 months, DAT SPECT imaging will be added. Annual visits are nested within the “Other SPECT Timepoint” selection options when submitting the data to Invicro in iPACS.

Table 2: Schedule of SPECT Assessments for Companion Study (Protocol 004)

Scan	Transitioning participants from Early Imaging 1.0 [a]	Newly enrolled participants for Early Imaging 2.0 [a]
Visit 02 (6 months)	X	X
Visit 04 (12 months)	X	-
Visit 05 (18 months)	X	X
Premature Withdrawal (PW)[b]	X	X

[a] PD participants transitioning from Early Imaging 1.0 and newly enrolled in Early Imaging 2.0 will be recruited to the Early Imaging 2.0 protocol (004). Participants transitioning from PPMI 001 to PPMI Clinical will continue from the PPMI 001 study schedule into the next planned study visit under PPMI Clinical.

[b] If a participant withdraws from the study and agrees to attend one more visit, the next scheduled annual visit should be completed. DAT SPECT imaging will be performed if not done in the previous 3 months.

Table 3: Schedule of Tau PET Assessments for Tau PET Study (008)

Scan	[^{18}F]PI-2620 PET Imaging [a]
Tau PET Imaging Visit	X

[a] Tau PET imaging can occur at any one of the annual or remote visits.

2.3 Previously-Acquired SPECT Scans for Protocol 002

2.3.1 Criteria for Previously-Acquired SPECT Scans

Previously-acquired DAT SPECT imaging for screening for Protocol 002 may be used if the following requirements have been met.

- The previously-acquired SPECT scan must have been acquired using the DaTscan radiotracer.
- A participant's previously-acquired DAT SPECT scan may be used in place of obtaining a newly acquired PPMI scan if the previous scan was acquired within 6 months of the study scheduled DAT SPECT Screening visit.
- The prior DAT SPECT scan must meet protocol acquisition standards and must pass the QC procedures established by Invicro. If the prior scan does not pass the QC procedures or cannot be reliably read by Invicro, the prior scan will not be considered valid, and the participant must undergo a newly acquired PPMI screening scan.
- Relevant prior data must be the original, raw scan images themselves, not the resulting clinical reading.

2.3.2 Transfer of Previously-Acquired SPECT Scans to Invicro

The clinical site will contact the SPECT imaging center where the previously-acquired SPECT data is maintained to coordinate the transfer of the participant's previously-acquired SPECT scan from the site's imaging center to Invicro.

Transfer of previously-acquired SPECT scan data and documentation to Invicro may be initiated as soon as a participant has provided full consent to participate in PPMI. Previously-acquired images will be transferred according to the approved methods detailed in the TOM.

2.3.3 Review of Previously-Acquired SPECT Scans

Previously-acquired scans will require the same centralized, Independent Review as SPECT images acquired during the trial as described in this Charter. If the previously-acquired DAT SPECT scan is considered acceptable, the participant does not need to undergo an additional DAT SPECT screening scan for trial inclusion.

Previously-acquired DAT SPECT imaging for screening for Protocol 002 may be used if the scan was acquired within 6 months of the study scheduled DAT SPECT Screening visit and meets protocol acquisition standards.

A previously-acquired DAT SPECT scan that has already been submitted to Invicro for another study, that meets the requirements for inclusion, will not need to be re-submitted through the iPACS portal. However, sites will need to complete a *PPMI Request for Previously-Acquired DaTscan* form for the AT SPECT imaging and provide it to the IND Site Management Core (SMC). This information will then be provided to Invicro.

A previously-acquired DAT SPECT scan that is not already in Invicro's database will need to be submitted through the iPACS portal and labeled as "Previously-Acquired / Screening" on the iPACS submission form.

A DAT SPECT scan that is acquired for two studies, and is not currently in the Invicro database, that meet the requirements for inclusion (including participant consent for both studies), will be submitted to the PPMI Clinical database through the iPACS portal and labeled as "Screening" on the iPACS submission form. Sites will also need to provide the other study's Subject ID and site number. This information can be provided to Invicro Project Management through email.

3 Image Data Management: Pre-Review

Image data archiving and QC will be strictly governed by Invicro's standard procedures. Invicro will track all submitted image data within the iPACS system. iPACS is a web-based 21CFR §11-validated data management system that supports data upload and storage processes.

3.1 Image Data Archiving

Invicro will ensure that images are de-identified and within a standardized file format before being saved in the database of images and presented during the Independent Review. All image data will

be tracked in an audit trail that tracks time-, date-, and user-stamps on digital data for each step within the image data management processes.

The data will be assessed and archived on a 21CFR §11-validated system. Data will be stored and backed up according to Invicro standard procedures. Invicro will implement daily incremental back-ups on physically and logically secure file servers. Data will be stored long-term in a secure, off-site facility for 25 years, unless otherwise specified by MJFF.

3.2 Image Data QC

All image data will be assessed for quality using Invicro modality-specific standard QC processes combined with trial-specific checks. QC will be performed using a 21CFR §11-validated system.

3.2.1 Receipt and Initial Checks

As soon as submitted image data are received at Invicro, the digital file identifiers will be cross-referenced against the submission form. If the digital file identifiers do not correspond, Invicro will run additional checks to ensure that the proper file has been identified (ie, checking the header for time and date of acquisition, and checking any other scan or study identifiers). Invicro will also compare the submission form with the submitted images. The following parameters will be reviewed and checks will be performed as part of the initial review:

- Name of file(s) received
- Confirmation of participant identity between images and submission form
- Clerical accuracy in the submission form
- Missing images
- Completeness of required series/sequences

3.2.2 Image QC

After the initial receipt and checks, an Imaging QC & Processing Specialist (or otherwise qualified Invicro employee) will perform a QC review of the image data to verify that the submission is compliant with trial-specific critical protocol parameters, the integrity of the image data has been maintained, and the image data is suitable for further review and analysis. The following parameters will be reviewed, and checks will be performed as part of the image QC

- Compliance with the imaging requirements
- Anatomical coverage
- Presence of artifacts that prevent accurate image interpretation

3.3 Image Transfer Agreement

Invicro and LONI will work to develop a trial-specific Image Transfer Agreement that outlines specifics of the image data to be transferred to LONI and designee, including the format of images, frequency of image transfer, and structure of the image data to be transferred.

4 SPECT Visual Assessment

4.1 Overview of Reviewer Assessment of SPECT

4.1.1 Presented Images

Each Reviewer will assess SPECT scans for participants newly enrolled in PPMI Clinical (Protocol 002) in the Healthy Control and PD cohorts that were acquired at the Screening Visit using either the ^{123}I-DaTscan™ radioligand or ^{99m}Tc-TRODAT-1. For protocol 002, a previously-acquired scan may be used to determine eligibility if a prospective participant has previously received a DAT SPECT scan, and all the requirements detailed in the protocol have been met.

4.1.2 Blinding

The images presented to the Reviewers will be blinded in the typical measures for image review, which includes the masking of participant demographics, participant treatment outcome, and all other clinical information not deemed essential for the Independent Review. Reviewers will be blinded to the identity of the of the sites and participants.

4.1.3 Scheduling

Assessment may begin after approval of the eCRF configuration is approved and the completion of Reviewer training. Invicro will schedule review sessions according to Reviewer schedules and the availability of participant images and related data. Review sessions will be scheduled in an ongoing basis.

4.1.4 Reviewer Training and Management

Invicro's standard procedures will govern the selection, training, re-training and ongoing evaluation of Reviewers' visual image assessments. Through this process, Reviewers must demonstrate initial and ongoing interpretative competency by reading training cases.

An expert nuclear medicine physician, trained and experienced in amyloid-targeting radiotracers, will train the Reviewers. Reviewers will be trained on approved radiotracer interpretation methods using the commercial training programs that align with the respective Prescribing Information (USPI), European Union Summary of Product Characteristics (SmPC), and/or by Invicro.

Ongoing inter-rater reliability will also be assessed by providing the Reviewers with various DATSPECT inter-rater cases in addition to standard clinical research cases. Remote inter-rater sessions will be organized to review a respective sampling of scans, and to discuss complex cases.

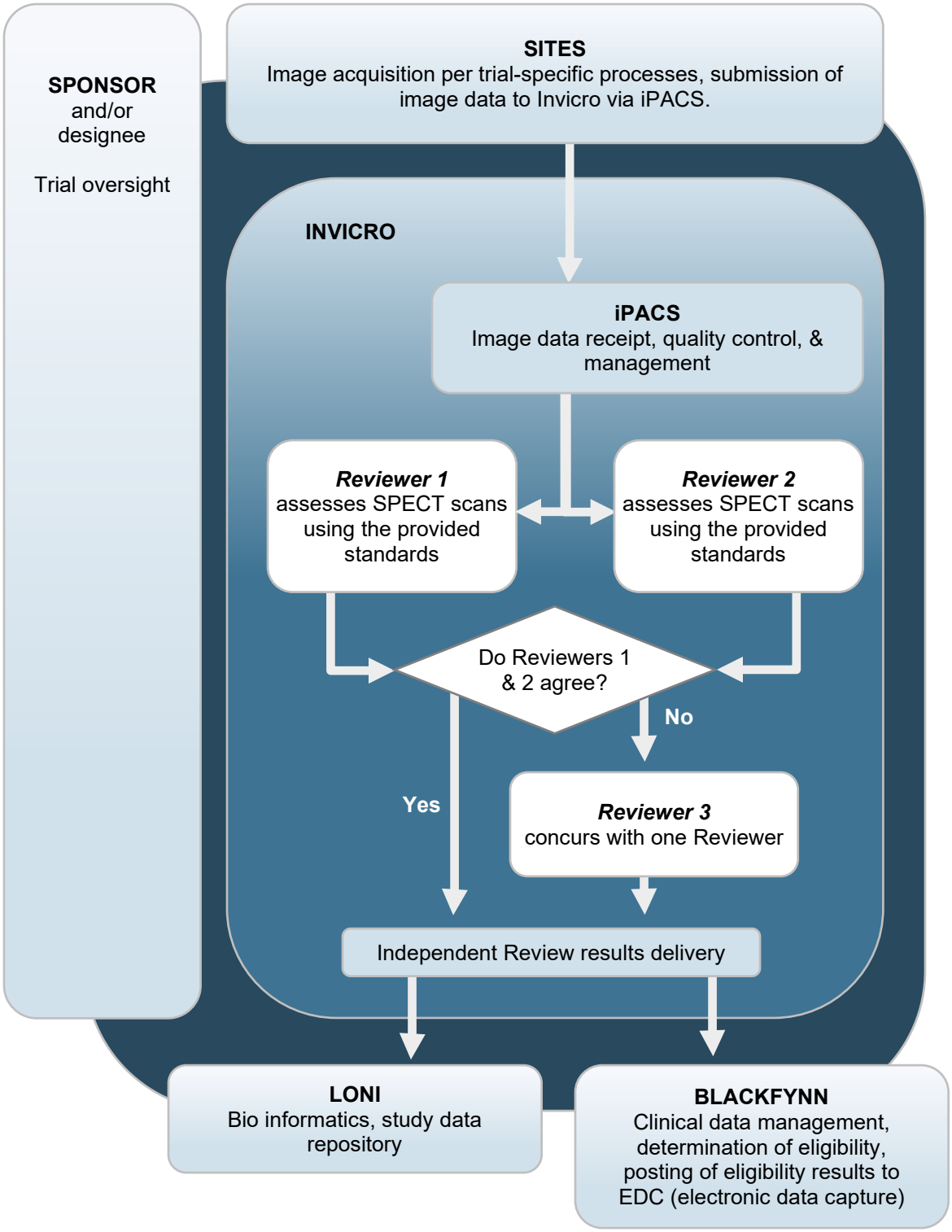
4.2 Visual Assessment Methods for SPECT

4.2.1 Reviewer Configuration

Two Reviewers will provide an overall visual interpretation of DAT SPECT scans for subjects in the PD or Healthy Cohort. If the 2 Reviewers' overall visual interpretations are discordant, a third Reviewer

will assess the images independently, and agreement of 2 out of 3 Reviewers will determine the overall interpretation of the scan. Figure 3 illustrates the Reviewer configuration and assessment sequence.

Figure 3: Visual Assessment of SPECT Configuration and Sequence



4.2.2 Software Application

Independent reviews will be conducted in PMOD, a biomedical image quantification software, which allows for audit trail with date-, time-, and user-stamps for any events during image access. The Reviewer will document the assessment on the DAT SPECT Visual Interpretation eCRF. The results of the assessment will be saved to iPACS.

4.2.3 SPECT Visual Assessment Criteria and Documentation

Each Reviewer will apply the tracer-specific criteria when assessing the DAT SPECT images. Each Reviewer will provide an overall assessment of *normal* (negative) or *abnormal* (positive) for radiotracer uptake, indicating normal or abnormal levels of striatal dopamine transporter levels respectively. Assessment will be documented on the SPECT Scan Visual Interpretation Form. Agreement of overall visual interpretation by 2 Reviewers (*normal* or *abnormal*) will determine the outcome of the scan for a given participant. Invicro will report the assessment to Blackfynn approximately 5 business days after receiving the DAT SPECT image data for eligibility scans.

4.2.4 SPECT Assessment Method

4.2.4.1 ^{123}I-DaTscan™

The following represents a portion of the instructions for image interpretation, which comes directly from the DaTscan™ (^{123}I-ioflupane injection) USPI.[5]

> ***Image Interpretation:***
>
> *DaTscan images are interpreted visually, based upon the appearance of the striata. Reconstructed pixel size should be between 3.5 and 4.5 mm with slices 1 pixel thick. Optimum presentation of the reconstructed images for visual interpretation is transaxial slices parallel to the anterior commissure-posterior commissure (AC-PC) line. Determination of whether an image is normal or abnormal is made by assessing*
>
> *the extent (as indicated by shape) and intensity of the striatal signal. Image interpretation does not involve integration of the striatal image appearance with clinical signs and/or symptoms.*
>
> ***Normal:***
>
> *In transaxial images, normal images are characterized by two symmetric comma- or crescent-shaped focal regions of activity mirrored about the median plane. Striatal activity is distinct, relative to surrounding brain tissue.*
>
> ***Abnormal:***
>
> *Abnormal DaTscan images fall into at least one of the following three categories (all are considered abnormal).*
>
> - *Activity is asymmetric, eg, activity in the region of the putamen of one hemisphere is absent or greatly reduced with respect to the other. Activity is still visible in the caudate nuclei of both hemispheres resulting in a comma or crescent shape in one and a circular or oval focus in the other. There may be reduced activity between at least one striatum and surrounding tissues.*
> - *Activity is absent in the putamen of both hemispheres and confined to the caudate nuclei. Activity is relatively symmetric and forms two roughly circular or oval foci. Activity of one or both is generally reduced.*
> - *Activity is absent in the putamen of both hemispheres and greatly reduced in one or both caudate nuclei. Activity of the striata with respect to the background is reduced.*

4.2.4.2 ^{99m}Tc-TRODAT-1

The SPECT Visual Assessment method for ^{99m}Tc-TRODAT-1 closely aligns with the general principles of ^{123}I-DaTscan™ assessment of the striata provided in Section 4.2.4.1, with the following difference: signal-to-noise properties are slightly lower for TRODAT-1 resulting in some cases in healthy volunteers of striatal uptake with a more globular appearance compared to the classic “comma” shape more usually noted.

- For similar reasons, the longitudinal time window for assessing striatal dopaminergic integrity over the course of illness is shorter, and the striata disappear faster into the higher background than is the case for Ioflupane (DaTScan) SPECT.
- There are fewer image artifacts with TRODAT-1 owing to the cleaner radioactive decay schema of ^{99}m-Tc compared to ^{123}-I.

5 SPECT Processing and Quantitative Analysis

The DAT SPECT images for screening and longitudinal scans for all cohorts acquired in studies 002 and 004 will be processed and normalized for quantitative analysis. An IPS is only qualified to perform the following steps after extensive training and approval by management. A senior IPS will periodically perform quality assurance by reviewing template placement procedures.

5.1 Blinding for SPECT Quantitative Analysis

Image processing and quantitative analysis will be performed with blinding to treatment status and clinical information; only the injected tracer dose and times of assay and injection will be known to the IPS (for proper calculation). The IPS will not be blinded to the order of the scans for the following reasons:

- The transformation matrix from the first scan will be used to normalize all the participant’s subsequent SPECT scans.
- The SPECT scan analysis will be performed continuously throughout the duration of the trial.

5.2 Preliminary SPECT Processing

5.2.1 Attenuation Correction and Filtering for SPECT

SPECT imaging files undergo attenuation correction to correct for count loss in deeper brain structures. Utilizing a contoured ellipse drawn around the periphery of the participant's anatomy an attenuation coefficient is applied. The attenuation coefficient used is specific to each site and is derived from the phantom data acquired during the site's technical qualification visit. Following attenuation correction, a six-millimeter Gaussian Smoothing filter will be applied to reduce noise in the image.

5.2.2 Co-registration and Normalization for SPECT

5.2.2.1 Screening SPECT scans

Within the Fusion Module in PMOD, attenuation corrected, filtered SPECT data are normalized to the Montreal Neurologic Institute (MNInst space).[6] For all SPECT normalization, the transformation matrices are saved as well as the resulting images.

5.2.2.2 Longitudinal SPECT scans

The longitudinal reconstructed SPECT data is co-registered rigidly to the screening reconstructed SPECT data using the Rigid Matching algorithm in PMOD. The IPS reviews the resulting image for suitable alignment. The resulting Rigid Matching transformation matrix is saved. The co-registered SPECT image is also saved.

Following co-registration, the co-registered longitudinal SPECT image will be attenuation corrected and filtered as described in Section 5.2.1. The IPS will utilize the ellipse generated during analysis of the screening scan to ensure that an identical attenuation mask is applied for a given participant across all timepoints.

The IPS then applies the normalization transformation matrix generated during analysis of the screening scan This will result in a SPECT image which is normalized to MNInst space and is in the same orientation as the screening image.

5.3 Quantitative Analysis of SPECT Scans

Quantitative analysis is performed in PMOD by applying regions of interest (ROIs) to images that have been normalized to MNInst space, and the 8 striatal slices with the greatest uptake are summed together to construct an image to use for analysis. A standardized SPECT ROI Template (Figure 4) is used to extract count densities from multiple brain regions (Table 4).

Figure 4: Standardized SPECT ROI Template

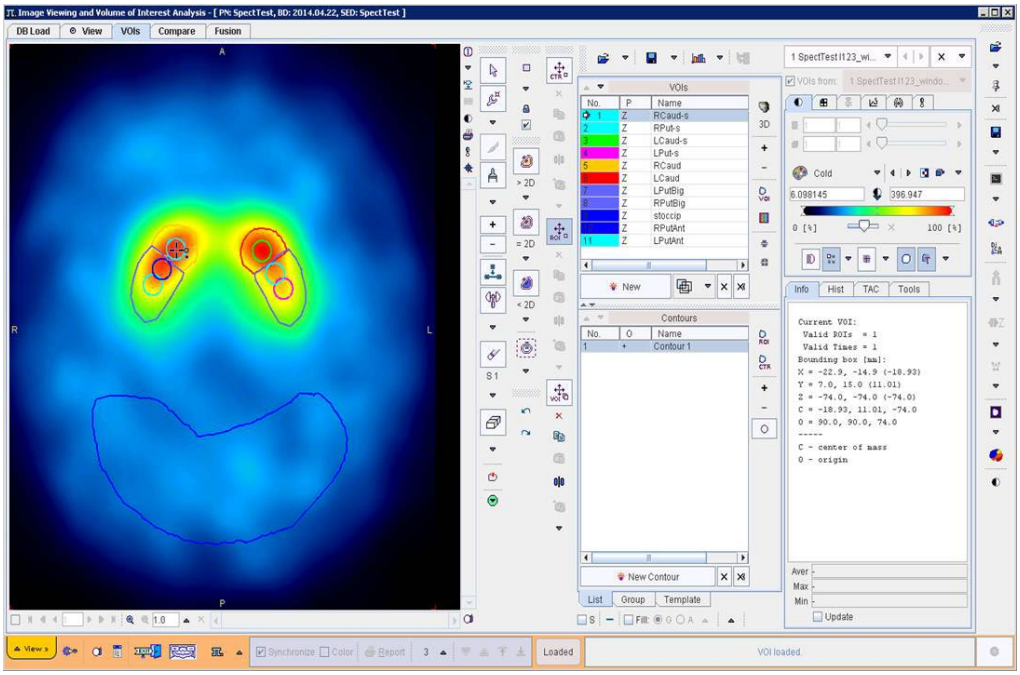


Table 4: Comprehensive Brain Regions for SPECT Quantitative Analysis

ROI Name	Analysis Template ROI Name [a]
RIGHT CAUDATE SMALL	RCaud-s
LEFT CAUDATE SMALL	LCaud-s
RIGHT PUTAMEN SMALL	RPut-s
LEFT PUTAMEN SMALL	LPut-s
RIGHT ANTERIOR PUTAMEN	RPutAnt
LEFT ANTERIOR PUTAMEN	LPutAnt
[a] Brain region name as shown in PMOD software	

5.3.1 Quantitative Extraction for SPECT

Once the template has passed QC, it is applied to the participant's normalized SPECT image for extraction of the values in the ROIs (Table 4). The outputs are saved as a text file that cannot be modified within PMOD.

5.3.2 SBR Determination for SPECT

The SBRs are calculated for the areas of interest using the calculation in Equation 1. For DAT SPECT images, the SBR values are calculated using the occipital cortex as a reference region. *Note, for scans*

where the standard occipital ROI is unable to be accurately placed resulting from anatomical (eg, atrophy, etc.) and/or technical (eg, subject positioning, etc.) factors, adjustments will be made to occipital ROI placement and/or an alternate reference region will be chosen with the recommendation from an expert Reviewer.

Equation 1: SBR Calculation

(Averaged (region of interest) – Averaged of the 'stoccip' region) / (Averaged of the 'stoccip' region)

6 Threshold Analysis for Eligibility for SPECT

The screening scans for the Healthy Control, PD, and Prodromal participants acquired in study 002 will be processed and quantitatively assessed by Invicro (see Section 5 for SPECT Processing and Quantitative Analysis). The SBR data for ROIs listed in Table 5 will then be transferred to Blackfynn who will calculate the threshold and apply a pre-determined algorithm to determine eligibility results.

Table 5. ROIs for Threshold Analysis of SPECT

ROI Name	Analysis Template ROI Name [a]
RIGHT CAUDATE SMALL	RCaud-s
LEFT CAUDATE SMALL	LCaud-s
RIGHT PUTAMEN SMALL	RPut-s
LEFT PUTAMEN SMALL	LPut-s
OCCIPITAL	stoccip
[a] Brain region name as shown in PMOD software	

For SBR threshold analysis, the minimum putamen (calculated as the minimum SBR value from either the left and right putamen) and average putamen (calculated as the mean SBR value of the combined left and right putamen) will be determined. The minimum putamen value (*minput*) will then be used, along with participant age at the time of the scan (*age*), to calculate a minimum putamen ratio which will utilize the formula in Equation 2.

Equation 2: Minimum Putamen Ratio

$$minput/(-0.0153 * age + 2.9576)$$

The average putamen ratio will be calculated similarly using the average putamen (*aveput*) and participant age at the time of the scan (*age*). This calculation will utilize the formula in Equation 3.

Equation 3: Average Putamen Ratio

$$aveput/(-0.0161 * age + 3.1187)$$

A participant will be deemed DAT eligible if at least one of the 2 VI scores is positive and either the minimum putamen ratio OR average putamen ratio is less than 0.80. Additional expert review will be performed on scans who do not meet the eligible criteria but fall in either of 2 categories:

- Two negative VI scores and either the minimum putamen ratio or average putamen ratio is less than or equal to 0.60
- Two positive VI scores and either the minimum putamen ratio or average putamen ratio is greater than or equal to 0.80.

If a scan does not meet the eligibility criteria or does not fall into either of the 2 categories for additional expert review, the scan will be deemed not eligible.

Note: The eligibility determination will be determined and delivered by Blackfynn.

7 Tau PET Quantitative Analysis

The Tau PET images for acquired at the single timepoint in study 008 will be processed and normalized for quantitative analysis. An IPS is only qualified to perform the following steps after extensive training and approval by management. A senior IPS will periodically perform quality assurance by reviewing template placement procedures.

7.1 Blinding for Tau PET Quantitative Analysis

Image processing and quantitative analysis will be performed with blinding to clinical information; only the injected tracer dose and times of assay and injection will be known to the IPS (for proper calculation).

7.2 Tau PET Smoothing

Specific to the Tau PET imaging, a smoothing step will be accomplished using convolution with a 3D Gaussian kernel. The Gaussian full width half maximum (FWHM) will be based on the phantom data that will be acquired during site set-up. FWHM should be selected to harmonize data across scanners and sites while maintaining consistently reconstructed spatial resolution

7.3 Tau PET Motion Correction

The IPS assesses all frames for patient movement in the X, Y, and Z planes and will run the PMOD function for Motion Correction. The IPS then re-assesses the frames for motion in the X, Y, and Z

planes. If Motion Correction was successful, the IPS appropriately saves the motion corrected file prior to further processing.

Note: In instances where there is more profound motion the IPS may opt to eliminate those frames to avoid inaccurate attenuation mapping. This frame removal is made feasible by the oversampling built into the Tau PET acquisition protocol. Data are eligible for analysis if one acceptable five-minute frame is present.

7.4 Tau PET Averaging

The motion corrected 5-minute emission frames are averaged to increase the signal-to-noise ratio. The IPS ensures that all frames for Tau PET are selected prior to running PMOD for this purpose. The IPS does a qualitative assessment of the averaging, and once confirmed, appropriately saves the file prior to further processing.

7.5 Tau PET Standard Uptake Value (SUV) Calculation

SUV will be calculated as follows:

Equation 4: SUV Calculation

$$SUV = \frac{C}{\left(\frac{D}{W}\right)}$$

C = tracer concentration measured from the image (in MBq/ml); D = injected tracer dose (MBq); W = total body weight (g) at the time of the scan; SUVs will be expressed in g/ml. The resulting SUV image will be saved for further processing as described in this section.

If metadata are insufficient for SUV calculation, the output concentration values will be saved in the original image units to enable downstream calculation of SUVR.

7.6 NEURO Module Workflow

The NEURO module within PMOD presents a processing workflow for registration of subject-specific Tau PET and MRI data to one another and to a common template space. The workflow can be executed as a manual pipeline in which it relies on the user to initiate and progress through each step; or the user can initiate the pipeline to run in a fully automated manner through the use of a protocol with pre-defined parameters and settings for each step. With the latter method, all QC review is performed by the IPS at the conclusion of the pipeline. The NEURO module will be used for all subsequent processing of both Tau PET and MRI data in which the following steps will be performed:

7.7 MRI Cropping

Cropping is used to limit the MRI to the brain (ie, removing shoulders, neck). The NEURO module will execute an automated cropping function in which it will generate a ROI that encompasses the brain, from the most inferior portion of the cerebellum to the apex of the skull. The IPS will view the cropped image to confirm that the entire brain is within the image field of view (FOV) and saves the cropped

MRI prior to further processing. If the outcome of the automated cropping is inadequate, the IPS will make manual adjustments as needed.

7.8 MRI Segmentation

Segmentation is a process performed on the T1-weighted, 3D volumetric MRI which identifies pixels of high signal intensity, such as white matter and/or pixels of low signal intensity, such as cerebrospinal fluid (CSF). The NEURO module separates the T1 weighted image into probability maps of gray matter, white matter and CSF to which a threshold is applied for generation of binary maps of each tissue type. The IPS reviews the resulting images to ensure proper segmentation. If adequate, the segmented images (gray matter, white matter and CSF maps) are saved. If an MRI segmentation should fail, the nearest temporal MRI scan with a successful segmentation will be used instead. In the rare event that MRI segmentation should fail for all MRI scans, due to inadequacy of the acquired T1-weighted MRI images (eg, extensive atrophy, motion during acquisition, artifacts present in the image), and no additional MRIs will be acquired, the Tau PET images will be co-registered to their respective non-segmented T1-weighted MRI image. Following normalization of the MRI and Tau PET images to standard space (MNInst space) ROIs will be placed on the non-segmented MRI. During this 'general' analysis the ROIs will be adapted to minimize the inclusion of white matter in gray matter regions, and vice versa.

7.9 Co-Registration and Normalization of Tau PET

Within the NEURO module, the Tau PET data sets are normalized to the Montreal Neurologic Institute (MNInst space);[6] the 4-step process described below transforms Tau PET data and subject-specific gray matter (GM) / white matter (WM) /CSF segmentation maps into a common template space. If Tau PET data are being analyzed using a previously processed MRI scan, then steps 2 and 3 are omitted.

1. The averaged Tau PET is co-registered rigidly to the cropped MRI using the Rigid Matching algorithm. The IPS reviews the resulting image for suitable alignment. The resulting Rigid Matching transformation matrix is saved. The co-registered Tau PET is also saved.
2. The cropped MRI is nonlinearly spatially normalized to the MNInst reference template using the probability maps normalization algorithm. The IPS reviews the resulting image for suitable alignment with the MNInst reference template. The resulting Brain Normalization transformation matrix is saved. This spatially normalized MRI is also saved.
3. The Brain Normalization transformation matrix (Step 2) is applied to the previously computed GM/WM/CSF segmentation maps. The result is a segmented MRI which is normalized to the MNInst space. The IPS reviews the resulting image for suitable alignment with the MNInst reference template. The normalized segmented MRI is saved.
4. The Rigid Matching transformation matrix (Step 1) and Brain Normalization transformation matrix (Step 2) are combined and applied to the average Tau PET image. The result is a Tau PET image which is normalized to the MNInst space and is in the same orientation as the subject's MRI. The IPS reviews the resulting image for suitable alignment with the MNInst reference template. This normalized Tau PET is saved.

7.10 Tau PET ROI Template Application and QC

The Hammers template (Figure 5) will be used in this study and regions in this template are listed in Table 6. Once the ROI template has been applied to the normalized images, the resulting template is saved and QC'd by the IPS. Template placement is always reviewed by image processing personnel for accuracy for reasons such as brain atrophy that result in inaccurate placement on subcortical structures, and to confirm the adequacy of the normalization to template space. All cortical and cerebellar ROIs are reviewed to ensure that the lateral edge of the ROI is aligned with the lateral edge of the brain in the Tau PET image. The subcortical structures are checked to ensure that the CSF is not within the delineated ROIs.

Figure 5: Hammers ROI Template for Tau PET Analysis

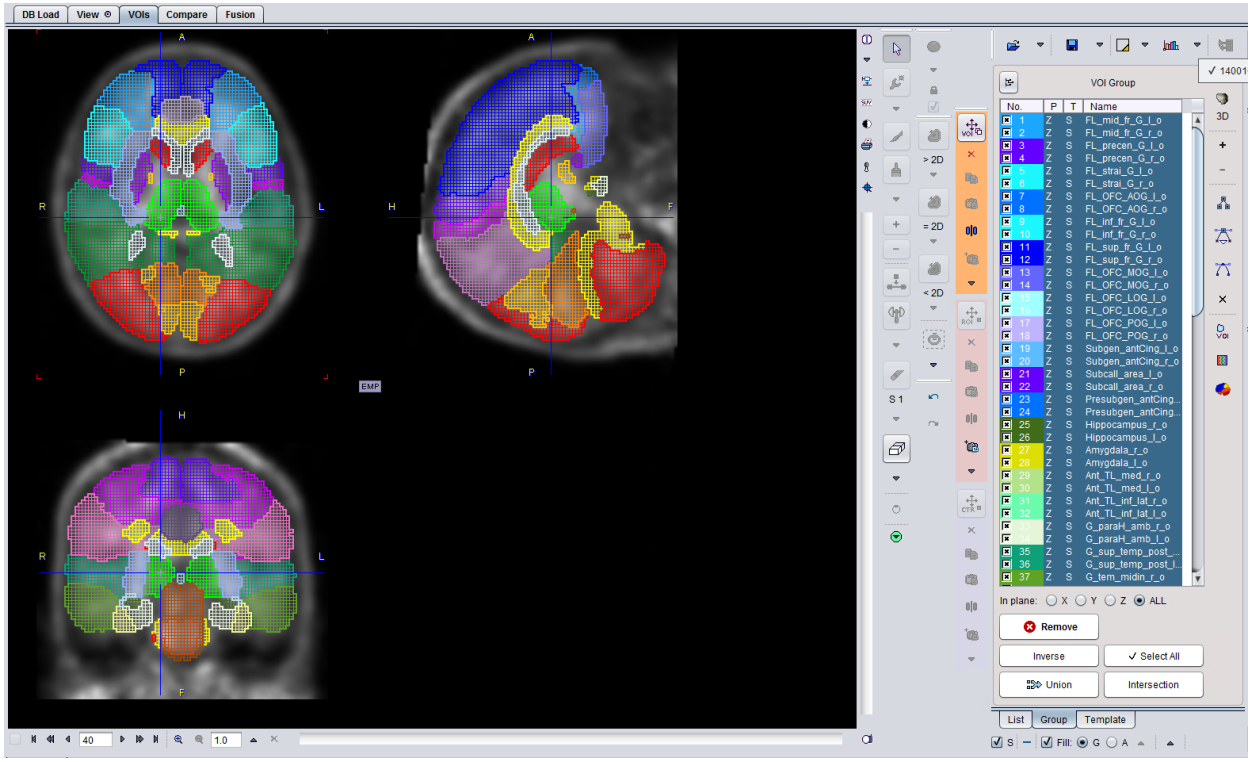


Table 6: Brain Regions List for Tau PET Analysis

PMOD Analysis Template ROI Name	ROI DESCRIPTION
FL_MID_FR_G_L	MIDDLE FRONTAL GYRUS LEFT
FL_MID_FR_G_R	MIDDLE FRONTAL GYRUS RIGHT
FL_PRECEN_G_L	PRECENTRAL GYRUS LEFT
FL_PRECEN_G_R	PRECENTRAL GYRUS RIGHT

PMOD Analysis Template ROI Name	ROI DESCRIPTION
FL_STRAI_G_L	STRAIGHT GYRUS LEFT
FL_STRAI_G_R	STRAIGHT GYRUS RIGHT
FL_OFC_AOG_L	ANTERIOR ORBITAL GYRUS LEFT
FL_OFC_AOG_R	ANTERIOR ORBITAL GYRUS RIGHT
FL_INF_FR_G_L	INFERIOR FRONTAL GYRUS LEFT
FL_INF_FR_G_R	INFERIOR FRONTAL GYRUS RIGHT
FL_SUP_FR_G_L	SUPERIOR FRONTAL GYRUS LEFT
FL_SUP_FR_G_R	SUPERIOR FRONTAL GYRUS RIGHT
FL_OFC_MOG_L	MEDIAL ORBITAL GYRUS LEFT
FL_OFC_MOG_R	MEDIAL ORBITAL GYRUS RIGHT
FL_OFC_LOG_L	LATERAL ORBITAL GYRUS LEFT
FL_OFC_LOG_R	LATERAL ORBITAL GYRUS RIGHT
FL_OFC_POG_L	POSTERIOR ORBITAL GYRUS LEFT
FL_OFC_POG_R	POSTERIOR ORBITAL GYRUS RIGHT
SUBGEN_ANTCING_L	SUBGENUAL FRONTAL CORTEX LEFT
SUBGEN_ANTCING_R	SUBGENUAL FRONTAL CORTEX RIGHT
SUBCALL_AREA_L	SUBCALLOSAL AREA LEFT
SUBCALL_AREA_R	SUBCALLOSAL AREA RIGHT
PRESUBGEN_ANTCING_L	PRE-SUBGENUAL FRONTAL CORTEX LEFT
PRESUBGEN_ANTCING_R	PRE-SUBGENUAL FRONTAL CORTEX RIGHT
HIPPOCAMPUS_L	HIPPOCAMPUS LEFT
HIPPOCAMPUS_R	HIPPOCAMPUS RIGHT
ANT_TL_MED_L	ANTERIOR TEMPORAL LOBE MEDIAL PART LEFT
ANT_TL_MED_R	ANTERIOR TEMPORAL LOBE MEDIAL PART RIGHT
ANT_TL_INF_LAT_L	ANTERIOR TEMPORAL LOBE LATERAL PART LEFT
ANT_TL_INF_LAT_R	ANTERIOR TEMPORAL LOBE LATERAL PART RIGHT
G_PARAH_AMB_L	PARAHIPPOCAMPAL AND AMBIENT GYRUS LEFT
G_PARAH_AMB_R	PARAHIPPOCAMPAL AND AMBIENT GYRUS RIGHT
G_SUP_TEMP_POST_L	SUPERIOR TEMPORAL GYRUS POSTERIOR PART LEFT
G_SUP_TEMP_POST_R	SUPERIOR TEMPORAL GYRUS POSTERIOR PART RIGHT
G_TEM_MIDIN_L	MIDDLE AND INFERIOR TEMPORAL GYRUS LEFT
G_TEM_MIDIN_R	MIDDLE AND INFERIOR TEMPORAL GYRUS RIGHT

PMOD Analysis Template ROI Name	ROI DESCRIPTION
G_FUS_L	FUSIFORM GYRUS LEFT
G_FUS_R	FUSIFORM GYRUS RIGHT
POST_TL_L	POSTERIOR TEMPORAL LOBE LEFT
POST_TL_R	POSTERIOR TEMPORAL LOBE RIGHT
G_SUP_TEMP_ANT_L	SUPERIOR TEMPORAL GYRUS ANTERIOR PART LEFT
G_SUP_TEMP_ANT_R	SUPERIOR TEMPORAL GYRUS ANTERIOR PART RIGHT
PL_POSTCE_G_L	POSTCENTRAL GYRUS LEFT
PL_POSTCE_G_R	POSTCENTRAL GYRUS RIGHT
PL_SUP_PA_G_L	SUPERIOR PARIETAL GYRUS LEFT
PL_SUP_PA_G_R	SUPERIOR PARIETAL GYRUS RIGHT
PL_REST_L	INFERIOLATERAL REMAINDER OF PARIETAL LOBE LEFT
PL_REST_R	INFERIOLATERAL REMAINDER OF PARIETAL LOBE RIGHT
OL_REST_LAT_L	LATERAL REMAINDER OF OCCIPITAL LOBE LEFT
OL_REST_LAT_R	LATERAL REMAINDER OF OCCIPITAL LOBE RIGHT
OL_LING_G_L	LINGUAL GYRUS LEFT
OL_LING_G_R	LINGUAL GYRUS RIGHT
OL_CUNEUS_L	CUNEUS LEFT
OL_CUNEUS_R	CUNEUS RIGHT
CAUDATENUCL_L	CAUDATE NUCLEUS LEFT
CAUDATENUCL_R	CAUDATE NUCLEUS RIGHT
PUTAMEN_L	PUTAMEN LEFT
PUTAMEN_R	PUTAMEN RIGHT
THALAMUS_L	THALAMUS LEFT
THALAMUS_R	THALAMUS RIGHT
G_CING_ANT_L	CINGULATE GYRUS ANTERIOR PART LEFT
G_CING_ANT_R	CINGULATE GYRUS ANTERIOR PART RIGHT
G_CING_POST_L	GYRUS CINGULI POSTERIOR PART LEFT
G_CING_POST_R	GYRUS CINGULI POSTERIOR PART RIGHT
SUBCORTICAL_WM	SUBCORTICAL WHITE MATTER
PONS	PONS
CEREBELLAR_WM	CEREBELLAR WHITE MATTER
CEREBELLUM_DORSAL_L	CEREBELLUM DORSAL EDGE LEFT

PMOD Analysis Template ROI Name	ROI DESCRIPTION
CEREBELLUM_DORSAL_R	CEREBELLUM DORSAL EDGE RIGHT
CEREBELLUM_VENTRAL	CEREBELLUM CORTEX
AMYGDALA_L	AMYGDALA LEFT
AMYGDALA_R	AMYGDALA RIGHT
NUCLACCUMB_L	NUCLEUS ACCUMBENS LEFT
NUCLACCUMB_R	NUCLEUS ACCUMBENS RIGHT
PALLIDUM_L	PALLIDUM LEFT
PALLIDUM_R	PALLIDUM RIGHT
CORP_CALLOSUM	CORPUS CALLOSUM
INSULA_L	INSULA LEFT
INSULA_R	INSULA RIGHT
BRAINSTEM	BRAINSTEM
MIDBRAIN	MIDBRAIN

Table 7: Composite Brain Regions for Tau PET Analysis

Composite Region Name	Regions Used In Calculation	Weighted/ Unweighted
BRAAK_1	G_SUP_TEMP_ANT_L G_SUP_TEMP_ANT_R G_PARAH_AMB_L G_PARAH_AMB_R	Weighted
BRAAK_2	HIPPOCAMPUS_L HIPPOCAMPUS_R	Weighted
BRAAK_3	AMYGDALA_L AMYGDALA_R G_FUS_L G_FUS_R OL_LING_G_L OL_LING_G_R	Weighted
BRAAK_4	ANT_TL_INF_LAT_L ANT_TL_INF_LAT_R G_TEM_MIDIN_L G_TEM_MIDIN_R INSULA_L	Weighted

Composite Region Name	Regions Used In Calculation	Weighted/ Unweighted
	INSULA_R G_CING_ANT_L G_CING_ANT_R G_CING_POST_L G_CING_POST_R POST_TL_L POST_TL_R	
BRAAK_5	G_SUP_TEMP_POST_L G_SUP_TEMP_POST_R OL_REST_LAT_L OL_REST_LAT_R FL_MID_FR_G_L FL_MID_FR_G_R PL_REST_L PL_REST_R FL_STRAI_G_L FL_STRAI_G_R FL_OFC_AOG_L FL_OFC_AOG_R FL_INF_FR_G_L FL_INF_FR_G_R FL_SUP_FR_G_L FL_SUP_FR_G_R PL_SUP_PA_G_L PL_SUP_PA_G_R FL_OFC_MOG_L FL_OFC_MOG_R FL_OFC_LOG_L FL_OFC_LOG_R FL_OFC_POG_L FL_OFC_POG_R SUBGEN_ANTCING_L SUBGEN_ANTCING_R	Weighted

Composite Region Name	Regions Used In Calculation	Weighted/ Unweighted
	PRESUBGEN_ANTCING_L PRESUBGEN_ANTCING_R	
BRAAK_6	FL_PRECEN_G_L FL_PRECEN_G_R PL_POSTCE_G_L PL_POSTCE_G_R OL_CUNEUS_L OL_CUNEUS_R	Weighted

7.11 Masking for Tau PET

The template is intersected with the subject-specific GM mask with some exceptions. Regions that are excluded from GM masking include hippocampus, amygdala, caudate, nucleus accumbens, putamen, thalamus, pallidum, corpus callosum, substantia nigra, brain stem, frontal horn, temporal horn, third ventricle, subcortical white matter, midbrain, and pons.

7.12 Tau PET Quantitative Extraction

Once the template has passed QC, it is applied to the subject's normalized Tau PET for extraction of the values in the ROIs. The extracted values are calculated as the mean of all voxels within each binary ROI mask. The outputs are saved as a text file that cannot be modified within PMOD.

7.13 Tau PET Standard Uptake Value Ratio (SUVR) Calculation

SUVRs will be computed using the ROIs listed in Table 7 compared with the CEREBELLUM_VENTRAL (CEREBELLAR CORTEX), which will serve as the reference. SUVRs are computed as the ratio of target regions to reference regions.

8 Data Management: Post-Review

Results generated from the Independent Review (both SPECT and Tau PET assessments) will be uploaded into the validated Invicro database, within iPACS. Once the Independent Review data have been loaded, they will be combined with relevant source data and extracted and formatted according to the trial-specific Data Transfer Agreements (DTAs) for final QC in preparation for outbound transfer.

8.1 Data Transfer Agreements (DTAs)

Invicro will work with the Blackfynn and LONI to prepare trial-specific DTAs to outline the transfer of the Independent Review database to Blackfynn and LONI, to be approved by all parties. The DTAs

will detail the following information as aligned with the project budget: data structure requirements, data transfer requirements (eg, the number, frequency and types [test, interim, final]), specific data variables, and methods of encryption and delivery. Complete details will be available in the approved DTAs.

8.2 Database Entry

Invicro's Image Analysis group will upload the PMOD output from the SPECT Quantitative Analysis into a trial-specific, validated database. Relevant source data (eg, time of injection, administration amount, participant weight, etc.) will be integrated, and the combined data will be extracted for final QC in preparation for delivery to Blackfynn and LONI on behalf of MJFF, according to the DTA.

8.3 Database Lock

Once all data requested by MJFF have been delivered and the final study report has been completed, Invicro will initiate the database closure process. Locking the database to read-only access will prevent any changes to the final database. A permanent copy of the stored Independent Review database is saved and cannot be altered.

8.4 Transfer to MJFF and/or Designee

Data transfer will be performed in accordance with the approved DTAs and the encryption and data security requirements communicated by MJFF or designee.

9 Supporting Documentation

9.1 SPECT and Tau PET Charter Amendment History

The table below is intended to summarize the changes from the previously approved final SPECT and Tau PET Charter. Revisions or amendments to the protocol will not require an amendment to the SPECT and Tau PET Charter, unless substantive changes impact review methods.

Version	Date	Summary of Changes
Final v1.0	30-Jul-2020	Original version
Final v2.0	21-Oct-2020	• Updated to include previously-acquired DaTscan images (protocol 002, Section 1.3.2, Section 2.2, Section 2.3, and Section 4.1) • Updated Invicro signatory • Editorial updates (global)
Final v3.0	28-Jan-2022	• Added Tau PET study (008) details (Section 1), Tau PET imaging (global), and Tau PET analysis (Section 7) • Clarified when processes apply to SPECT and/or Tau PET imaging (global) • Added ^{99m}Tc-TRODAT-1 as a SPECT radiotracer (global) • Updated based on Protocol 002 Version 1.2 (global) • Added Anterior Putamen to ROI List (Table 4) • Editorial Updates (global)

9.2 Abbreviations

21CFR §11	Title 21 Code of Federal Regulations Part 11— Electronic Records; Electronic Signatures
Charter	Independent Review Charter
CSF	Cerebrospinal fluid
DAT	Dopamine transporter
DTA	Data Transfer Agreement
eCRF	electronic Case Report Form
FDA	United States Food and Drug Administration
FOV	Field of view
FWHM	(Gaussian) full width half maximum
GBA	Glucocerebrosidase gene
GCP	Good Clinical Practice
GM	gray matter
IND	Institute for Neurodegenerative Disorders
iPACS	Invicro's data management system
IPS	Image Processing Specialist
LONI	Laboratory of Neuro Imaging
LRRK2	Leucine-rich repeat kinase 2
MJFF	Michael J. Fox Foundation
MNInst	Montreal Neurologic Institute
MRI	Magnetic resonance imaging
PD	Parkinson's disease
PET	Positron emission tomography
PMOD	Biomedical image quantification software
PPMI	Parkinson's Progression Markers Initiative
QC	Quality control
RBD	REM Sleep Behavior Disorder
ROI	Region of Interest
SBR	Striatal binding ratio
SmPC	European Union Summary of Product Characteristics
SNCA	Alpha-synuclein
SPECT	Single-photon emission computed tomography

SUV	Standardized uptake value
SUVR	Standardized uptake value ratio
TOM	Technical Operations Manual
USPI	United States Prescribing Information
WM	White matter

10 References

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