Independent Review Charter: SPECT

Document Version	Final v1.0; 30-Jul-2020
Sponsor for Main Study	Michael J. Fox Foundation
Main Study	
Protocol Identifier	002
Protocol Version	Version 1.1; 23-Jun-2020
Protocol Description	The Parkinson's Progression Markers Initiative (PPMI) 2.0 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)

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
Document Version	Final v1.0; 30-Jul-2020
Protocol Identifier	002 and 004

DocuSigned by Ken Marek
Ken Marek | I have reviewed this document
05-Aug-2020 | 5:29 AM PDT
B68E9EE01CA243DB883A885DB8BFCFFF

05-Aug-2020 | 5:29 AM PDT

Ken Marek, MD
Distinguished Scientist
Institute for Neurodegenerative Disorders

Date (dd-MMM-yyyy)

DocuSigned by John Seibyl
John Seibyl | I approve this document
11-Aug-2020 | 3:50 AM CDT
AFAF1747D6DE48CDBA5665AED202165B

11-Aug-2020 | 3:50 AM CDT

John Seibyl, MD
Distinguished Scientist
Institute for Neurodegenerative Disorders

Date (dd-MMM-yyyy)

Invicro 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
Document Version	Final v1.0; 30-Jul-2020
Protocol Identifier	002 and 004

DocuSigned by Edward Hogan
Edward Hogan
I approve this document
05-Aug-2020 | 6:23 AM CDT
2305F6FDC34142F7821A14EFC6CAA84F

05-Aug-2020 | 6:23 AM CDT

Edward Hogan, MS
Executive Vice President, Clinical Operations
Invicro, A Konica Minolta Company

Date (dd-MMM-yyyy)

DocuSigned by Jacob Hesterman
I approve this document
05-Aug-2020 | 6:17 AM CDT
B2CC59F5207B42AC92171E771A8654DB

05-Aug-2020 | 6:17 AM CDT

Jacob Hesterman, PhD
Head of Research and Development
Invicro, A Konica Minolta Company

Date (dd-MMM-yyyy)

DocuSigned by Jennifer Lowery
Jennifer Lowery
I approve this document
04-Aug-2020 | 2:30 PM EDT
1394260943E148A9AD43C026BB665054

04-Aug-2020 | 2:30 PM EDT

Jennifer Lowery
Senior Coordinator, Medical Writing
Invicro, A Konica Minolta Company

Date (dd-MMM-yyyy)

Table of Contents

1 Executive Summary

The 002 and 004 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, a Konica Minolta Company (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 and 004. 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 Independent Review Charter (SPECT Charter) must be reviewed and approved by MJFF.

1.2 Purpose and Scope of the Independent Review Charters

The purpose of the SPECT 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 Charter should be considered supplementary to the protocol 002.

The scope of the SPECT Charter outlines the activities necessary to conduct the Independent Review, specific to SPECT 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 positron emission tomography (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 sub-sets, 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 mutations (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 mutations [such as Parkin or Pink1]) and individuals with prodromal Parkinson disease (including individuals with RBD, olfactory loss, a LRRK2, GBA, SNCA or rare genetic mutations [such as Parkin or Pink1]) and/or other risk factors for PD with and without DAT deficit in comparison to healthy controls or in sub-sets 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 mutations (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 mutations [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 mutations (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 mutations (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 mutations (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 2.0 Online and PPMI 2.0 Remote studies.

The PPMI 2.0 study will include up to 4,500 participants at 30-40 international clinical sites. Participants will be in one of the following cohorts:

- Current PPMI 1.0 participants will be divided into the following cohorts:
    - Healthy Control
    - PD
    - Prodromal
- Newly enrolled PPMI 2.0 participants will be divided into the following cohorts:
    - Healthy Control
    - PD
    - Prodromal

For the main study (002), participants will receive up to 4 DaTscan™ SPECT scans, depending on the cohort designation. DaTscan SPECT scans will be used to evaluate disease progression through imaging biomarkers. DaTscan SPECT 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 DaTscan 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 six months thereafter.

For the companion study (004), participants will have up to 3 DaTscan™ SPECT scans, depending on if the participant is transitioning from PPMI 1.0 to 2.0 or newly enrolled in PPMI 2.0. DaTscan 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 positron emission tomography (PET), including the imaging schedule and analysis, refer to the approved Quantitative Analysis Methodology: [^{18}F] AV-133 for study 004.

1.3.2 Role of SPECT Imaging in the Trial

1.3.2.1 Eligibility

Eligibility for participants newly enrolled in PPMI 2.0 will be determined through qualitative and quantitative assessments of screening DaTscan SPECT images. Current PPMI 1.0 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.

1.3.2.2 Longitudinal Changes in Imaging Biomarkers

SPECT imaging will support the study outcomes for longitudinal changes in quantitative DaTscan 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 Function of the SPECT Independent Review

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

- **DaTscan 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.
- **DaTscan 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.

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

1.4 Roles and Responsibilities

1.4.1 MJFF

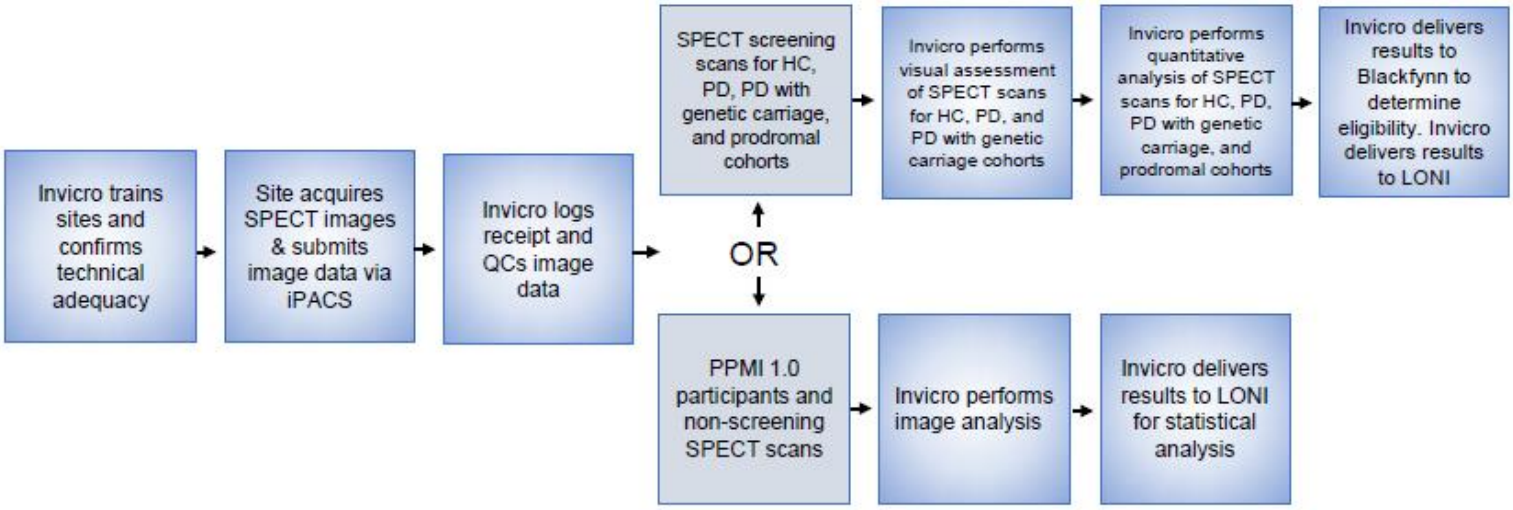
MJFF and designated contract research organization(s) are responsible for the overall conduct of the trial, including protocol design, regulatory compliance, clinical site designation, imaging center designation, all contracting and payment, regulatory and ethics committee submissions, participant recruitment, 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, A Konica Minolta Company

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

Figure 1: Overview of the SPECT 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 Charter development
- Site interaction
  - Distribution of the SPECT Technical Operations Manual (TOM)
  - Technical site set-up for SPECT 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 DaTscan SPECT Visual Assessment, contracting with those candidates

- Configuration of the Independent Review database and the electronic case report form (eCRF) that captures assessment results
- Pre-processing and quantitative analysis of DaTscan SPECT performed by an Image Processing Specialist (IPS)
- Post-review quality checks
- Delivery of Independent Review results database for DaTscan SPECT Eligibility to Blackfynn
- Delivery of Independent Review results database to Laboratory of Neuro Imaging (LONI)
- Preparation of the final study report

1.4.3 Independent Reviewers

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 the site investigator and 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 a modality-specific TOM for SPECT scans to the participating sites. The TOM communicates the trial-specific process standards designed to control for variability during image acquisition. The TOM includes 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)
- QC performed by the site personnel
- Site training and qualification process
- Schedule of imaging assessments
- Instructions for image archiving
- 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.

2.2 Scheduled Imaging

Sites will be instructed to acquire SPECT imaging according to the Schedule of Assessments (Table 1 and Table 2), as stipulated by the protocols, and according to the parameters in the SPECT TOM. Sites will submit these images to Invicro for inclusion in the Independent Review and quantitative analysis.

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

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

[a] Cohorts include participants newly enrolled in PPMI 2.0 and participants transitioning from PPMI 1.0. Newly enrolled participants in PPMI 2.0 will start with the Screening Visit. Participants transitioning from PPMI 1.0 to PPMI 2.0 will not have a screening DaTscan, and these participants will start at the equivalent visit in PPMI 2.0 from the PPMI 1.0 schedule.

[b] DaTscan™ screening imaging appointments will occur within 60 days prior to Day 1.

[c] Participants in the Genetic PD Cohort with LRRK2 or GBA mutations may not have the following drugs within 6 months of the Screening Visit: dopamine receptor blockers (neuroleptics), metoclopramide and reserpine.

[d] DaTscan™ imaging will occur within ±45 days of the scheduled visit.

[e] If a participant withdraws from the study and agrees to attend one more visit, the next scheduled annual visit should be completed. DaTscan™ imaging will be performed if not done in the previous 6 months.

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)[d]	X	X
Visit 04 (12 months)	X	-
Visit 05 (18 months)[d]	X	X
Premature Withdrawal (PW)[e]	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). Newly enrolled participants in PPMI 2.0 will start at Visit 02. Participants transitioning from PPMI 1.0 to PPMI 2.0 will continue from the PPMI 1.0 study schedule into the next planned study visit under PPMI 2.0.

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

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 DaTscan SPECT Visual Assessment

4.1 Overview of Reviewer Assessment

4.1.1 Presented Images

Each Reviewer will assess SPECT scans for participants newly enrolled in PPMI 2.0 in the Healthy Control and PDcohorts that were acquired at the screening visit using the DaTscan radioligand.

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.

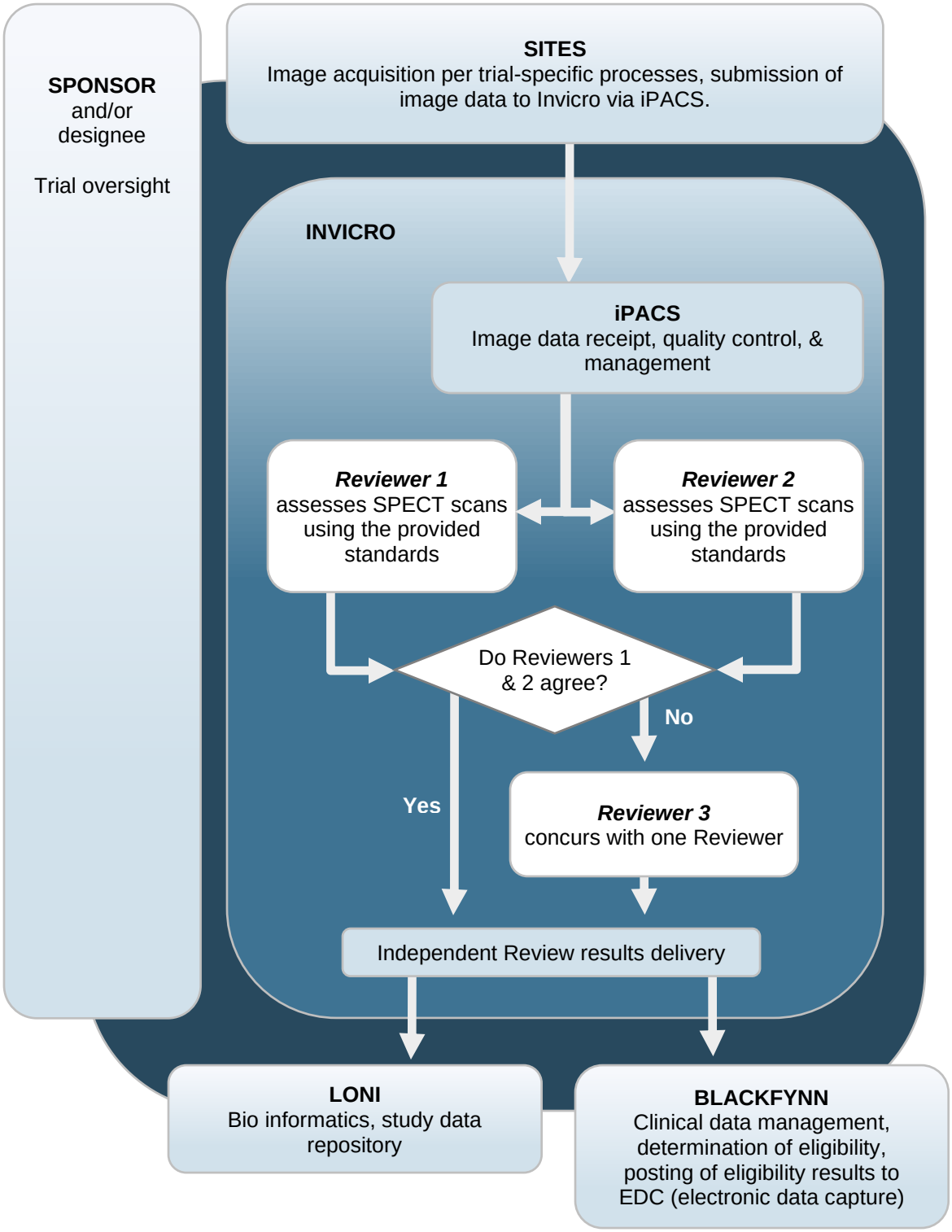
Ongoing inter-rater reliability will also be assessed by providing the Reviewers with various DaTscan SPECT inter-rater cases in addition to standard clinical research cases. Remote inter-rater sessions will be organized to review a respective sampling of scans, and to discuss complex cases.

4.2 Visual Assessment Methods

4.2.1 Reviewer Configuration

Two Reviewers will provide an overall visual interpretation of DaTscan SPECT scans. 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 2 illustrates the Reviewer configuration and assessment sequence.

Figure 2: Independent Review 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 DaTscan SPECT Visual Interpretation eCRF. The results of the assessment will be saved to iPACS.

4.2.3 Visual Assessment Criteria and Documentation

Each Reviewer will apply the tracer-specific criteria when assessing the DaTscan 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 DaTscan SPECT image data for eligibility scans.

4.2.4 Assessment Method

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.*

5 SPECT Processing and Quantitative Analysis

The DaTscan 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 demonstration of standardized placement through inter-rater assessment and approval by management. Inter-rater assessment is carried out at annual intervals (or more frequently as needed). To date, intra-class correlations have been >0.90 for all assessed raters using these segmentation methods. A senior IPS will periodically perform quality assurance by reviewing template placement procedures.

5.1 Blinding for 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

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 anthropomorphic 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

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 eight striatal slices with the greatest uptake are summed together to construct an image to use for analysis. A standardized SPECT ROI Template (Figure 3) is used to extract count densities from multiple brain regions (Table 3).

Figure 3: Standardized SPECT ROI Template

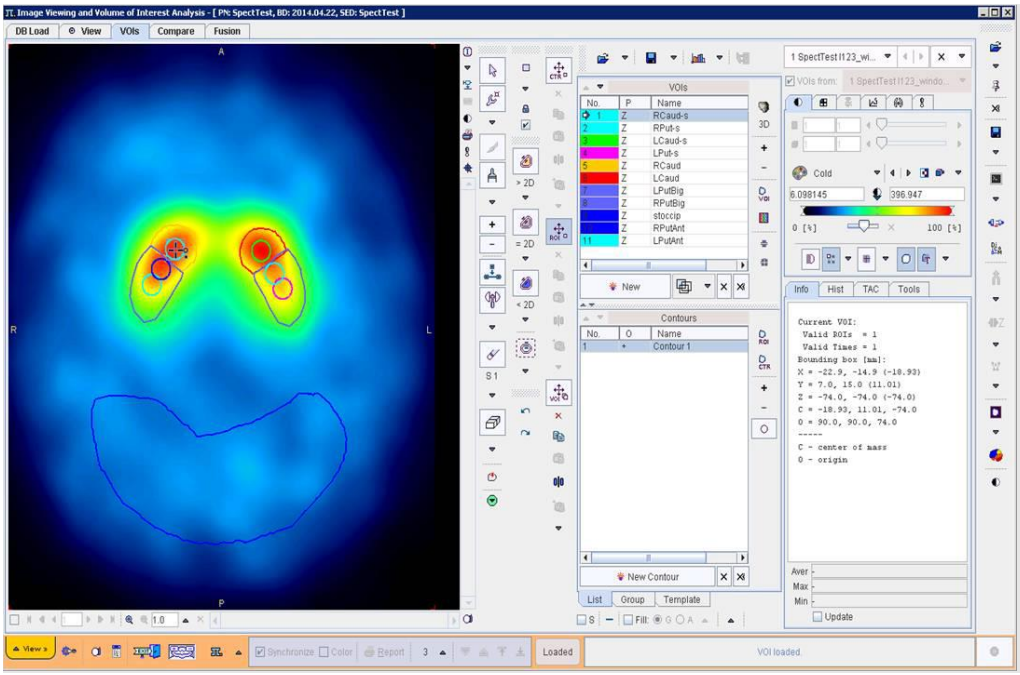


Table 3: Comprehensive Brain Regions for SPECT Quantitative Analysis

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

5.3.1 Quantitative Extraction

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 3). The outputs are saved as a text file that cannot be modified within PMOD.

5.3.2 SBR Determination

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

Equation 1: SBR Calculation

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

6 Threshold Analysis for Eligibility

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 4 will then be transferred to Blackfynn who will calculate the threshold and apply a pre-determined algorithm to determine eligibility results.

Table 4. ROIs for Threshold Analysis

ROI Name [a]	Analysis Template ROI Name
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 two 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 two 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 Data Management: Post-Review

Results generated from the Independent Review will be uploaded from PMOD 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.

7.1 Data Transfer Agreements

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.

7.2 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.

7.3 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.

7.4 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.

8 Supporting Documentation

8.1 SPECT Charter Amendment History

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

Version	Date	Summary of Changes
Final v1.0	30-Jul-2020	Original version

8.2 Abbreviations

21CFR §11	Title 21 Code of Federal Regulations Part 11— Electronic Records; Electronic Signatures
Charter	Independent Review Charter
DAT	Dopamine transporter
DTA	Data Transfer Agreement
eCRF	electronic Case Report Form
FDA	United States Food and Drug Administration
GBA	Glucocerebrosidase gene
GCP	Good Clinical Practice
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
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
TOM	Technical Operations Manual
USPI	United States Prescribing Information

9 References

1. International Council for Haromisation of Technical Requirements for Pharmaceuticals for Human Use. E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1): Guidance for Industry. In:March 2018: https://www.fda.gov/downloads/Drugs/Guidances/UCM464506.pdf. Accessed March.

2. United States Food and Drug Administration, Department of Health and Human Services. Clinical Trial Imaging Endpoint Process Standards: Guidance for Industry. In:April 2018: https://www.fda.gov/downloads/drugs/guidances/ucm268555.pdf.

3. United States Food and Drug Administration, Department of Health and Human Services. Guidance for Industry: Computerized Systems Used in Clinical Investigations. In:2007.

4. United States Food and Drug Administration, Department of Health and Human Services. Title 21 Code of Federal Regulations Part 11: Electronic Records; Electronic Signatures. In:1997: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=11. Accessed Jan 24, 2019.

5. DaTscan (Ioflupane I123 injection) [prescribing information]. In. Chicago, IL: GE Healthcare; 2015.

6. Senjem ML, Gunter JL, Shiung MM, Petersen RC, Jack CR, Jr. Comparison of different methodological implementations of voxel-based morphometry in neurodegenerative disease. *Neuroimage.* 2005;26(2):600-608.