ClinicalTrials.gov ID: NCT04594941

STATISTICAL ANALYSIS PLAN **GE Healthcare**
GE-265-001

Title: A Descriptive, Comparative, Randomized, Crossover Study of Flurpiridaz (^{18}F) Injection for Positron Emission Tomography (PET) Imaging for Assessment of Myocardial Perfusion Imaging Quality using High Performance Liquid Chromatography (HPLC) and Solid Phase Extraction (SPE) Manufacturing Processes

This Statistical Analysis Plan was prepared by the following personnel:

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Title [redacted]
Company [redacted]

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REVISION HISTORY

Version	Date	Changes implemented
1.0	[redacted]	Not applicable.
2.0	Refer to the signature page.	Updates to presentation of subject disposition table. Clarification of time of recording for medical history and baseline periods for clinical laboratory evaluation.

ABBREVIATIONS

AE	Adverse event
BMI	Body mass index
CAD	Coronary artery disease
CI	Confidence Interval
ECG	Electrocardiogram
eCRF	Electronic case report form
HPLC	High Performance Liquid Chromatography
IMP	Investigational medicinal product
ITT	Intent-to-treat
MedDRA	Medical Dictionary for Regulatory Activities
MITT	Modified intent-to-treat
MMRM	Mixed-effects model for repeated measures
MPI	Myocardial perfusion imaging
PET	Positron emission tomography
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SPE	Solid Phase Extraction
SRS	Summed rest score
SUV	Standard uptake value
TAC	Time-activity curve
TEAE	Treatment-emergent adverse event
WHO-DD	World Health Organization Drug Dictionary

1 INTRODUCTION

Doses of Flurpiridaz (^{18}F) Injection used in the nonclinical and clinical studies performed by Lantheus Medical Imaging were manufactured using high performance liquid chromatography (HPLC) purification. This process is also being used to manufacture doses of Flurpiridaz (^{18}F) Injection for the current GE Healthcare clinical study (GE-265-303). The HPLC method is not ideal for future routine manufacturing of [^{18}F]fluorine tracers. GE Healthcare has, therefore, developed the manufacturing process for Flurpiridaz (^{18}F) Injection on the FASTlab synthesis module, a current and supported synthesis platform. This process enables the production of Flurpiridaz (^{18}F) Injection batches with approximately 4 times the product radioactivity compared to the HPLC process. This will significantly improve the availability of Flurpiridaz (^{18}F) Injection. In addition, the solid phase extraction (SPE) manufacturing process on FASTlab is expected to enhance control, consistency and production reliability because it eliminates the manual cut required for HPLC purification. Also, it uses single-use commercially available cartridges, reagents and other disposable materials supplied in a preassembled cassette that is assembled, controlled and released by GE Healthcare under current Good Manufacturing Practice. The drug product from the SPE manufacturing process will have improved compatibility with dispensing equipment at manufacturing sites and pharmacy syringes. The latter is important for hospitals to use their standard radiation protection equipment to ensure low radiation dose for hospital staff. It is also important to avoid errors in dosing with use of non-compatible syringes.

Study GE-265-001 is a descriptive, comparative, randomized, crossover study of Flurpiridaz (^{18}F) Injection for positron emission tomography (PET) imaging for assessment of myocardial perfusion imaging (MPI) quality using HPLC and SPE manufacturing processes.

The statistical analysis plan (SAP) will provide details to further elaborate statistical methods as outlined in the protocol (GE-265-001 Clinical Protocol Amendment A01, Approved 03 Feb 2022) and will describe analysis conventions to guide the statistical programming work. The SAP will be signed off before the study database is locked.

Exploratory analyses, not identified in this SAP, may be performed to support the clinical development program. Any post-hoc, or unplanned, analyses that are performed but not identified in this SAP will be clearly identified in the clinical study report.

2 STUDY OBJECTIVES, DESIGN AND PROCEDURES

2.1 Objectives

Primary Objective:

- Assess difference and variability between 2 sets of visually-based global rest scores resulting from 2 at-rest MPI sessions using Flurpiridaz (^{18}F) Injection doses synthesized by the same or 2 manufacturing processes.

Secondary Objectives:

- Assess the intra-reader agreement of the detection of ischemic defect on PET-MPI at rest between 2 MPI acquisitions using 2 Flurpiridaz (^{18}F) Injection doses.
- Assess the detection of myocardial segmental defect similarity from 2 sets of images acquired from doses synthesized by the same or 2 different manufacturing processes of Flurpiridaz (^{18}F) Injection PET for MPI at rest.
- Assess the equivalence of 2 sets of [^{18}F]flurpiridaz time-activity curves (TACs) (blood, myocardium, lungs, liver) resulting from MPI using Flurpiridaz (^{18}F) Injection doses synthesized by the same or 2 different manufacturing processes.
- Assess the safety of Flurpiridaz (^{18}F) Injection PET doses synthesized by 2 different manufacturing processes.

2.2 Study Design

This is a Phase 2 prospective, randomized, crossover study of Flurpiridaz (^{18}F) Injection for PET-MPI in subjects referred for evaluation of known coronary artery disease (CAD) or for suspected CAD with intermediate to high pre-test probability.

This study will be conducted at approximately 4 centers in the US. Twenty-eight evaluable subjects will be enrolled. Assuming a 20% drop-out rate, approximately 34 subjects will need to be enrolled initially. At least 30% of subjects enrolled should have a documented history of MI, defined by history of a fixed defect.

Each subject will attend a Screening Visit at least 2 days and up to 14 days prior to the first Flurpiridaz (^{18}F) Injection PET-MPI (PET-MPI Visit 1). The investigator will explain what participation in the study entails and check to determine that the subject meets all the inclusion criteria but none of the exclusion criteria.

Subjects will undergo 2 Flurpiridaz (^{18}F) Injection PET-MPI procedures at rest performed no more than 2 weeks apart. To allow for adequate decay of the first dose, at least 24 hours must separate the 2 injections/imaging procedures. The subjects will be randomized 1:1:1:1 to 4 possible sequences of receiving 2 doses of Flurpiridaz (^{18}F) Injection: 7 subjects will receive 2 Flurpiridaz (^{18}F) Injection doses synthesized by the same HPLC manufacturing process (HPLC → HPLC), 7 subjects will receive 2 Flurpiridaz (^{18}F) Injection doses synthesized by the

same SPE manufacturing process (SPE → SPE), and 14 subjects will receive 2 Flurpiridaz (^{18}F) Injection doses synthesized by 2 different manufacturing processes (7 subjects will receive 1 dose manufactured by HPLC followed by 1 dose manufactured by SPE (HPLC → SPE) and 7 subjects will receive 1 dose manufactured by SPE followed by 1 dose manufactured by HPLC (SPE → HPLC)). All doses will be administered at rest by bolus intravenous injection in a large peripheral vein.

The targeted dose to the body of Flurpiridaz (^{18}F) Injection will be in the range of 1.7 to 2.5 mCi (63 to 93 MBq) for each administration and will not exceed a total of 6 mCi (222 MBq) for an individual subject. The image acquisitions in a list-mode at a single bed position centered on the heart will start 10 seconds before Flurpiridaz (^{18}F) Injection and last 15 minutes. In effort to avoid bias in image acquisition, the test product manufacturing status will not be specified on the syringe.

All subjects will be followed up by telephone for adverse events (AEs) and serious AEs (SAEs) at 24 (+8) hours following each Flurpiridaz (^{18}F) Injection administration.

Therefore, study subjects will participate in the study for up to 30 days.

A subject will be considered to have completed the study if he/she has completed all phases of the study including the last scheduled procedure shown in the Study Schedule of Events (Table 1).

An overview of study procedures is presented in Figure 1. A schedule of study procedures is provided in Table 1.

Figure 1 Study Diagram

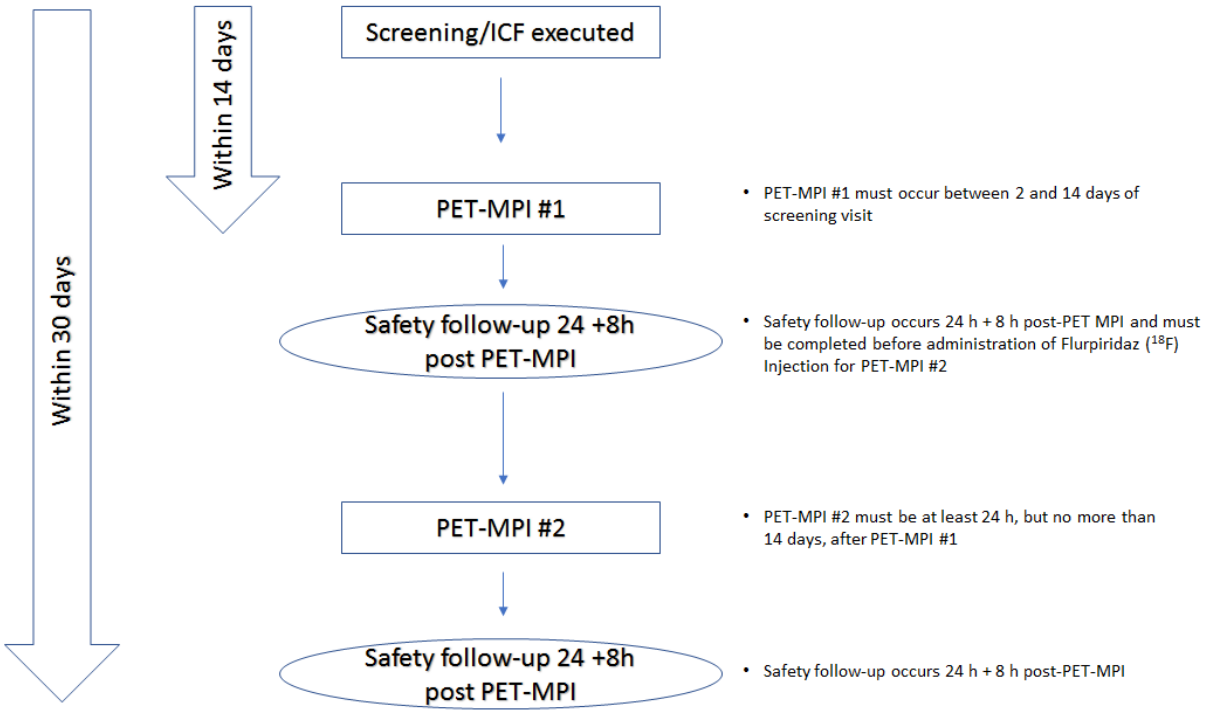

Table 1 Study Schedule of Events

Variables	Screening	PET-MPI Visit 1 (2 to 14 days after Screening)	Safety Follow-up (24+8 hours after PET-MPI Visit 1)	PET MPI Visit 2 (≥24 hours and <2 weeks after PET-MPI Visit 1)	Safety Follow-up (24 + 8 hours after PET-MPI Visit 2)
Informed consent	X				
Entry criteria	X				
Pregnancy test [a]	X	X		X	
Demographic information	X				
Medical/surgical history	X				
Randomization	X				
Blood sampling	X	X[b]		X[b]	
Urine sampling	X	X[b]		X[b]	
Prior/concurrent medications	X	X	X	X	X
Vital signs and pulse oximetry	X	X[c]		X[c]	
12-lead ECG recording	X[d]	X[d]		X[d]	
Physical examination [e]		X[e]		X[e]	
Clinical screen for new/worsening symptoms		X[f]		X[f]	
Injection site monitoring		X[g]		X[g]	
IMP administration		X		X	
PET-MPI Image acquisition		X		X	
Adverse events (including AEs and SAEs)[h]	X	X	X	X	X

AE = adverse event; ECG = electrocardiogram; IMP = investigational medicinal product; MPI = myocardial perfusion imaging; PET = positron emission tomography; SAE = serious adverse event

* Safety follow-up after PET-MPI Visit 1 must be complete before administration of Flurpiridaz (18F) Injection for PET-MPI Visit 2.

a) For women of childbearing potential, the results of either a urine or serum human chorionic gonadotropin pregnancy test (with the result known on the day of and before radiopharmaceutical administration) must be negative.

b) Before the IMP injection (blood and urine) and 1 hour after the IMP injection (blood).

c) Vital signs and pulse oximetry will be recorded up to 20 minutes pre-injection and at 30 (±5) minutes post-injection. In addition, heart rate only will be recorded at approximately 1 minute pre-injection and at 5, 10 and 15 minutes post-injection.

d) 12 lead ECG collected on local ECG machine and interpreted by the investigator or a designee at the investigative site. A clinically indicated ECG performed within 48 hours prior to screening, without intervening episodes of chest pain or instability, can be used as the Screening ECG, where available. At rest PET-MPI visits, 12-lead ECG will be recorded at pre-dose (within 20 minutes before transmission scan) and at 10 (±5) and 30 (±5) minutes post-injection.

e) Physical examination will comprise a full examination with a specific focus on cardiovascular signs. At rest PET-MPI visits, physical examination will be performed pre-injection and at 1 hour post-injection.

f) A clinical screen for new or worsening symptoms indicative of unstable CAD disease will be conducted prior to performing any procedures at the PET-MPI visits. If the clinical status screening is positive, the PET-MPI visits can either be rescheduled (up to 1 time and within the 2-week period specified) after symptoms stabilize or the subject can be discontinued from the study.

g) Immediately prior to injection, immediately after injection of IMP and 1 hour after injection of IMP.

h) All serious and non-serious AEs will be collected from the time of informed consent and followed for a final outcome until the end of the follow-up period.

2.3 Sample Size Calculation

A sample size of 28 subjects will be assessed for the clinical imaging similarity and safety profiles of Flurpiridaz (^{18}F) Injection synthesized by 2 different manufacturing processes in subjects with suspected or established CAD. To account for 20% dropout, approximately 34 subjects will be randomized in a 1:1:1:1 ratio to each of the 4 treatment sequences (SPE → SPE, HPLC → HPLC, SPE → HPLC, HPLC → SPE).

2.4 Interim Analysis

No formal interim analyses will be performed in this study.

2.5 Randomization and Blinding

Randomization:

This is a randomized crossover study. Twenty-eight evaluable subjects will be randomized 1:1:1:1 into 4 treatment sequence groups:

- Group 1: 2 doses of Flurpiridaz (^{18}F) Injection manufactured by the HPLC method (HPLC → HPLC)
- Group 2: 2 doses of Flurpiridaz (^{18}F) Injection manufactured by the SPE method (SPE → SPE)
- Group 3: 1 dose of Flurpiridaz (^{18}F) Injection manufactured by the HPLC method followed by 1 dose of Flurpiridaz (^{18}F) Injection manufactured by the SPE method (HPLC → SPE)
- Group 4: 1 dose of Flurpiridaz (^{18}F) Injection manufactured by the SPE method followed by 1 dose of Flurpiridaz (^{18}F) Injection manufactured by the HPLC method (SPE → HPLC)

Once subjects have completed the screening process they will be enrolled in the study and will be randomly assigned to one of the above 4 treatment sequences in accordance with a prespecified randomization list. Allocation to the enrollment groups, including randomization, will be performed centrally (via the IWRS in the EDC) by the contract research organization (CRO). No subject will be administered Flurpiridaz (^{18}F) Injection before it has been determined that he/she meets the study's inclusion criteria, does not meet the exclusion criteria, and signed and dated informed consent has been obtained. If any early withdrawal takes place for a subject (e.g., a subject only completes the first injection), the EDC/IWRS will assign the next subject to the same dosing sequence in order to make sure the design (1:1:1:1) is balanced at the end of study.

Blinding

This is a Phase 2, randomized study. Subjects and clinical site personnel will be blinded to the manufacturing process used to obtain Flurpiridaz (^{18}F) Injection. In effort to avoid bias in image acquisition, the test product manufacturing status will not be specified on the syringe.

The independent image readers will be blinded to subject number, medical history and the manufacturing process used to synthesize Flurpiridaz (^{18}F) Injection.

3 STUDY ENDPOINTS

3.1 Primary Efficacy Endpoint

- Intra-reader correlation and difference between the summed perfusion rest scores (SRS) and summed rest percent (SR%) after 2 MPI sessions using 2 Flurpiridaz (^{18}F) Injection doses synthesized using the same or 2 different manufacturing processes.

3.2 Secondary Efficacy Endpoints

- Variability of the SRS after MPI sessions using 2 Flurpiridaz (^{18}F) Injection doses synthesized for the subjects receiving 2 doses of the product manufactured by the SPE process.

- Variability of the SRS after MPI sessions using 2 Flurpiridaz (^{18}F) Injection doses synthesized for the subjects receiving 2 doses of the product manufactured by the HPLC process.

- Intra-reader agreement of the detection of ischemic defect on PET-MPI at rest between 2 MPI acquisitions using 2 Flurpiridaz (^{18}F) Injection doses.

- Difference between the perfusion rest scores for each of the 17 segments and each reader using 2 Flurpiridaz (^{18}F) Injection doses synthesized using the same or 2 different manufacturing processes.

- Difference in the standard uptake value (SUV) TACs and the relative difference in SUV (5- to 15-minute perfusion image) in left ventricular cavity, myocardium, lungs, and liver measured after two [^{18}F]flurpiridaz injections using doses of Flurpiridaz (^{18}F) Injection synthesized by 2 different manufacturing processes.

- Difference in the SUV TACs and the relative difference in SUV (5- to 15-minute perfusion image) in left ventricular cavity, myocardium, lungs, and liver measured after two [^{18}F]flurpiridaz injections using doses of Flurpiridaz (^{18}F) Injection for the subjects receiving 2 doses of the product manufactured by the HPLC process.

- Difference in the SUV TACs and the relative difference in SUV (5- to 15-minute perfusion image) in left ventricular cavity, myocardium, lungs and liver measured after two [^{18}F]flurpiridaz injections using doses of Flurpiridaz (^{18}F) Injection for the subjects receiving 2 doses of the product manufactured by the SPE process.

- Intra-reader agreement of the image quality score between the 2 sets of PET images acquired after two [^{18}F]flurpiridaz injections using doses of Flurpiridaz (^{18}F) Injection.

3.3 Image Acquisition

Imaging procedures include PET-MPI.

The image acquisitions in a list-mode at a single bed position centered on the heart will start 10 seconds before Flurpiridaz (^{18}F) Injection and last 15 minutes. Both PET images must be acquired on the same PET camera.

The PET list-mode acquisition will be reframed to produce a dynamic series for TAC analysis (0 to 15 minutes) and a static image (5 to 15 minutes) with and without cardiac gating for visual and semiquantitative analysis. Full details of the imaging protocol are presented in the GE-265-001 PET Imaging Manual.

Incidental findings are not likely.

3.4 Image Interpretation

Three qualified independent readers will perform a blinded assessment of each subject's PET image pair (with each injection images). Further details will be provided in the GE-265-001 PET Independent Review Charter.

Details of the TAC and SUV analysis will be provided in the GE-265-001 PET Image Processing Manual. Each subject will have a test and retest perfusion series. The uptake statistics from the liver, lung, left ventricle, and myocardium (17-Segment, vascular territories and whole) will be generated.

3.5 Safety Endpoints

Subjects will be closely monitored for safety until completion of all study procedures. Safety monitoring will include AEs, medication errors, treatment-emergent AEs (TEAEs), and SAE assessments, vital signs, ECGs, hematology, clinical chemistry laboratory tests, and urinalysis. All subjects will be followed up by telephone for AEs at 24 (+8) hours following each Flurpiridaz (^{18}F) Injection administration. The 24-hour safety follow-up after PET-MPI Visit 1 must be completed before administration of Flurpiridaz (^{18}F) Injection for PET-MPI Visit 2.

The investigator(s) and the Sponsor/CRO will review the safety data. The following safety data will be collected and evaluated:

- Clinical laboratory parameters: serum biochemistry, hematology and urinalysis (Table 2).
- Vital signs: systolic/diastolic blood pressure, heart rate, respiration rate, and pulse oximetry.
- 12-lead ECG.
- Physical examination.
- Injection site monitoring.
- Post-treatment events (AEs and SAEs).

Pre-specified normal limits for vital signs and ECG intervals are provided in Section 6.6.

Table 2 Clinical Laboratory Parameters

Serum Biochemistry	Hematology	Urinalysis
Alanine aminotransferase (ALT) Albumin Aspartate aminotransferase (AST) Bicarbonate Bilirubin (total) Calcium Chloride Creatinine Gamma-glutamyltransferase Glucose Lactate dehydrogenase Potassium Protein (total) Sodium Urea nitrogen	Hematocrit Platelet count White blood cell (WBC) count	Bilirubin Glucose Ketone Occult blood pH Protein Urobilinogen

4 ANALYSIS POPULATIONS

4.1 Intent-to-Treat (ITT) Population

The ITT population will consist of all subjects randomized to receive ≥1 dose of Flurpiridaz (^{18}F) Injection in the study.

4.2 Modified Intent-to-Treat (MITT) Population

The MITT population will include all ITT subjects who have completed the 2 rest Flurpiridaz (^{18}F) Injection PET-MPI procedures. The MITT population will be the primary analysis set for the primary efficacy endpoint.

4.3 Safety Population

The Safety Population will include all subjects who have received ≥1 dose of Flurpiridaz (^{18}F) Injection in the study. All safety data will be summarized for the Safety Population.

5 ANALYSIS CONVENTIONS

Post-text tables and listings will be prepared in accordance with the ICH M2 Guidelines [ICH 2008]. The information and explanatory notes to be provided in the "footer" or bottom of each table and listing will include the following information:

1. Date and time of output generation;
2. SAS® program name, including the path that generates the output;
3. Any other output-specific details that require further elaboration.

In general, tables will be formatted with columns displaying findings based on the study arm for all subjects. The summary tables will clearly indicate the number of subjects to which the data apply, and *unknown* or *not performed* are distinguished from *missing* data.

Supportive individual Subject Data Listings, as a minimum, will be sorted and presented by the study arm, subject number, and visit date, if applicable.

This section details general conventions to be used for the statistical analyses. The following conventions will be applied to all data presentations and analyses.

- Summary statistics for categorical variables will consist of the number and percentage of responses in each level. The number and percentage of responses will be presented in the form XX (XX.X%).
- Summary statistics for continuous variables will consist of the sample size (n), mean, median, standard deviation (SD), minimum, and maximum values.
- All mean and median values will be formatted to one more decimal place than the measured value. SD values will be formatted to two more decimal places than the measured value. Minimum and maximum values will be presented with the same number of decimal places as the measured value.
- All p-values, if applicable, will be rounded to 4 decimal places. All p-values that round to 0.0000 will be presented as '<0.0001' and p-values that round to 1.0000 will be presented as '>0.9999'. One-sided P-values <0.025 will be considered to be statistically significant unless otherwise specified.
- All summary tables will include the analysis population sample size (i.e., number of subjects).
- Study Day 1 is defined as the date at which the subject received their first dose of investigational medicinal product (IMP). All study days are determined relative to Day 1.
- Study days prior to Day 1 will be calculated as:

- Study Day = Assessment Date – Date first IMP dose received

- Study days after Day 1 will be calculated as:
  - Study Day = Assessment Date – Date first IMP dose received + 1.
- Baseline values will be defined as the last non-missing value recorded prior to first IMP dose received.
- Change from baseline will be calculated as follows:
  - Change = Post-baseline value - baseline value.
- All pre- and post-enrolment assessments including unscheduled or repeat assessments will be included in the data listings.
- Date variables will be formatted as YYYY-MM-DD for presentation.
- Tables, figures, and listings will be presented in landscape orientation.
- SAS® Version 9.4 or higher will be the statistical software package used for all data analyses.

5.1 Definition of Analysis Windows

There are no visit windows for this study. For the statistical analyses, data will be analyzed by the nominal visit that was collected on the electronic case report form (eCRF).

Unscheduled visits will not be used in the by-visit analysis but will be used for the following where appropriate: 1) derivations of baseline/last on-treatment measurements; 2) derivations of the maximum/minimum on-treatment values and maximum/minimum changes from baseline values for safety analyses; 3) data listings.

5.2 Definition of Missing Data Imputation

Missing values will not be substituted by estimated values but treated as missing in the statistical evaluation. All data from all subjects dosed in the study will be included in all listings, plots, summary tables, and statistical analyses when appropriate.

5.3 Handling of Missing Data/Censoring/Discontinuation

To explore the extent of missing data, a summary of baseline characteristics will be created to compare groups of subjects with non-missing results with those who have missing results to assess if there is a significant difference between the 2 groups. This summary will be performed separately in ITT subjects who are missing PET MPI results.

5.4 Handling of Uninterpretable Images

Once a subject has completed the 2 rest Flurpiridaz (^{18}F) Injection PET-MPI procedures and is included in the MITT population, the readers will be asked to read PET-MPI images and to classify the images as normal or abnormal, regardless of image quality and interpretability.

5.5 Protocol Deviations

Any deviation from the protocol when no approved amendment exists must be documented as a protocol deviation and reported according to local requirements. If appropriate, corrective, and preventative action must be implemented to avoid repetition. Protocol deviations and any potential impact on the study results will be discussed during the reporting of the study and finalized before database lock.

Waivers or protocol exceptions will not be granted prospectively by the Sponsor under any circumstances.

6 STATISTICAL ANALYSES

In order to ensure the randomization is properly performed, summary for baseline characteristics will be presented by 4 sequences. Because 2 manufacturing processes may result in different impurity profiles, safety analyses will be summarized and presented by HPLC process and SPE process. For the primary efficacy endpoint of SRS and SR% and drug exposure will be summarized and presented by HPLC method and SPE method.

6.1 Subject Disposition

A table will be provided with the following information by sequence group and overall:

- Number of subjects enrolled.
- Number of subjects randomized.
- Number of subjects included in the efficacy analysis populations (ITT, MITT).
- Number of subjects included in the safety analysis population.
- Number of subjects withdrawn from the study and the reason for withdrawal. The number of subjects withdrawn from Period 1 (after the first dose) and from Period 2 (after the 2nd dose) will be summarized as well.

6.2 Demographics, Baseline Characteristics, Medical History, and Prior/Concomitant Medications

6.2.1 Demographic and Baseline Characteristics

Demographic information (age, height, weight, and body mass index [BMI]) will be summarized by using descriptive statistics. Sex, ethnicity, and race will be summarized by counts and percentages in MITT and safety population.

6.2.1 Medical/Surgical History

The subjects' relevant medical and surgical history as recorded at Visit 1 prior to IMP injection will be summarised, and the data will be presented using body systems and conditions/diagnoses as captured on the eCRF. The body systems will be presented in alphabetical order, and the conditions/diagnoses will be presented in alphabetical order within each body system.

The number and percentage of subjects with a particular condition/diagnosis will be summarized in the MITT and safety population. Subjects reporting more than one condition/diagnosis within a body system will be counted only once for that body system.

6.2.2 Prior and Concomitant Medications

Prior and concomitant medications are defined relative to Flurpiridaz (^{18}F) Injection administration. Prior and concomitant medications will be summarized in the MITT and safety population by counts and percentages using the World Health Organization Drug Dictionary (WHO-DD), and grouped by primary and secondary classes, if applicable. The version of WHO-DD used will be detailed in the footnotes of related tables, figures, and listings.

A prior medication is defined as any medication taken prior to the start of the first dose of Flurpiridaz (^{18}F) Injection. A *concurrent* medication is a new medication started after the start of the first dose of Flurpiridaz (^{18}F) Injection or a prior medication that continues after Flurpiridaz (^{18}F) Injection. A medication is defined as concurrent for Period 1 if it is taken on the day of Period 1 Flurpiridaz (^{18}F) Injection dosing. A medication is defined as concurrent for Period 2 if it is taken on the day of Period 2 Flurpiridaz (^{18}F) Injection dosing.

If a medication is taken prior to Flurpiridaz (^{18}F) Injection administration for Period 1 and no information on its use is available after the imaging, the medication will be assumed to be ongoing and therefore considered as both prior and concurrent medications. If the medication is taken concomitantly with Flurpiridaz (^{18}F) Injection administration and no information is available on its start date relative to Flurpiridaz (^{18}F) Injection administration, the medication will be considered as both prior and concurrent medications. Concomitant medications will be categorized to Period 1 or Period 2 based on the onset of the concomitant medication compared the time/date of study drug of each period.

Table 3 Classification of prior and concurrent medications

End date / Start date	Before start of IMP administration	On or after start of IMP administration	Missing
Before start of IMP administration	Prior	Prior/Concurrent	Prior/Concurrent
On or after start of IMP administration	–	Concurrent	Concurrent
Missing	Prior	Prior/Concurrent	Prior/Concurrent

6.3 Study Drug Exposure

Treatment exposure (Flurpiridaz (^{18}F) Injection dose in mCi or MBq) and volume administered will be summarized by descriptive summary statistics. Summary statistics will be presented by each dose. A summary of each subject's dosing information will be presented in a listing. Effective dose will be calculated as the product of the dose/unit activity and the administered activity. The targeted dose to the body Flurpiridaz (^{18}F) Injection will be in the range of 1.7 to 2.5 mCi (63 to 93 MBq) for each administration and will not exceed a total of 6 mCi (222 MBq) for an individual subject.

6.4 Primary Efficacy Analyses

6.4.1 Efficacy Variables

6.4.1.1 Myocardial Perfusion Imaging Evaluations

Three qualified readers (independent from the study) will perform independent reads of all MPI images, based on standard 17-segment polar-maps of perfusion defects. Each reader will score the perfusion pattern in each segment (17 segments) using a 5-point scale scoring:

- Normal 0,
- Minimal, mild impairment of perfusion, ambiguous image 1,
- Moderate impairment of perfusion 2,
- Significant impairment of perfusion 3,
- No perfusion 4.

The summed score of the 17 segments SRS, as well as the SR%, will be used as the primary endpoint, and summarized as a continuous variable by reader and using the median of the results of the 3 readers. SR% is calculated by dividing the SRS by a number corresponding to the SRS value indicating a deficit of 4 in every segment (68 points for 17 segments) and then multiplying the result by 100%.

6.4.2 Statistical Hypothesis, Model, and Method of Analysis

The primary analysis for the primary endpoint will be based on the mixed-effects model for repeated measures (MMRM). The model will include the median SRS in each dosing period as the dependent variable, with manufacturing process, period, and dosing sequence as fixed effects, and subject nested within sequence as the random effect. The estimated mean SRS difference and 90% confidence interval (CI) will be provided from the MMRM analysis.

Similarity will be established if the 90% CI of the difference is within a margin of (-0.75, 0.75). The margin size is defined as 0.75 to allow the difference of, at most, 43% of the mean SRS score (mean PET SRS score is 1.75 based on empirical data [Berman et al. 2013]).

The correlation analysis will be stratified by the following 3 groups:

1) subjects who have received 2 doses of product manufactured by 2 different processes.

2) subjects who have received 2 doses of product both manufactured by the SPE process.

3) subjects who have received 2 doses of product both manufactured by the HPLC process.

The intra-reader (within-subject) correlation between the 2 manufacturing processes will be assessed by Pearson's correlation, Kendall's τ and Bland-Altman difference for the summed score stratified by the above 3 groups. The mean of paired difference between the 2 manufacturing processes, t-statistics based 95% CI, the median difference and 95% CI based on Hodges-Lehman method, and p-value obtained by Wilcoxon signed rank test will also be

provided for descriptive purpose for the SRS among subjects who have received 2 doses of product manufactured by 2 different processes for each reader.

Sensitivity analyses will be performed to assess the impact of missing data on the primary analysis results (i.e., subjects in the ITT population with incomplete PET-MPI scans) using similar MMRM analysis for primary analysis.

6.4.3 Reader Difference

Reader variability will be assessed by the reread of images. Intra-reader reproducibility will be determined based on the re-read of images from approximately 25% of the randomly selected exams (not subjects). The same images will be re-read by all readers.

Intra-reader reproducibility assessed for both original read and re-read images by calculating Cohen's pairwise kappas and their respective 95% confidence intervals.

6.5 Secondary Efficacy Analyses

6.5.1 Secondary Efficacy Endpoint Analyses

Secondary efficacy endpoints include:

- Variability of the SRS after MPI sessions using 2 Flurpiridaz (^{18}F) Injection doses synthesized for the subjects receiving 2 doses of the product manufactured by the SPE process.

- Variability of the SRS after MPI sessions using 2 Flurpiridaz (^{18}F) Injection doses synthesized for the subjects receiving 2 doses of the product manufactured by the HPLC process.

- Intra-reader agreement of the detection of ischemic defect on PET-MPI at rest between 2 MPI acquisitions using 2 Flurpiridaz (^{18}F) Injection doses.

- Difference between the perfusion rest scores for each of the 17 segments and each reader using 2 Flurpiridaz (^{18}F) Injection doses synthesized using the same or 2 different manufacturing processes.

- Difference in the SUV TACs and the relative difference in SUV (5- to 15-minute perfusion image) in left ventricular cavity, myocardium, lungs and liver measured after two [^{18}F]flurpiridaz injections using doses of Flurpiridaz (^{18}F) Injection synthesized by 2 different manufacturing processes.

  The difference in the SUV TACs between 2 manufacturing processes will be measured using the Euclidean distance [Pini et al. 2022] from 2 TAC curves.

$$d(i) = \sqrt{\Delta t \sum_{t=t_1}^{t_r} (Y_{1i}(t) - Y_{2t}(t))^2}$$

Where $\Delta = \frac{tr - t1}{T}$

Difference in the SUV TACs between 2 different manufacturing processes will be compared using a nonparametric method of the sign test for testing no difference between 2 manufacturing processes.

- Difference in the SUV TACs and the relative difference in SUV (5- to 15-minute perfusion image) in left ventricular cavity, myocardium, lungs and liver measured after two [^{18}F]flurpiridaz injections using doses of Flurpiridaz (^{18}F) Injection for the subjects receiving 2 doses of the product manufactured by the HPLC process.

  Difference in the SUV TACs between the 2 HPLC processes will be measured using Euclidean distance and will be compared using a nonparametric method of the sign test for testing no difference between HPLC process from Period 1 and Period 2.

- Difference in the SUV TACs and the relative difference in SUV (5- to 15-minute perfusion image) in left ventricular cavity, myocardium, lungs and liver measured after two [^{18}F]flurpiridaz injections using doses of Flurpiridaz (^{18}F) Injection for the subjects receiving 2 doses of the product manufactured by the SPE process.

  Difference in the SUV TACs between the 2 SPE processes will be measured using the Euclidean distance and will be compared a nonparametric method of the sign test for testing no difference between SPE process from Period 1 and Period 2.

- Intra-reader agreement of the image quality score between the 2 sets of PET images acquired after two [^{18}F]flurpiridaz injections using doses of Flurpiridaz (^{18}F) Injection.

6.5.2 Secondary Endpoint Analyses

The intra-reader variability of the SRS will be estimated by reader using the SD of the paired difference divided by $\sqrt{2}$, for subjects receiving 2 doses of the product both manufactured by the SPE process and separately for subjects receiving 2 doses of the product both manufactured by the HPLC process.

For the individual scores of 17 segments by each reader, the mean of paired difference between the 2 manufacturing processes, t-statistics based 95% CI, the median difference and 95% CI based on Hodges-Lehman method, and p-value obtained by Wilcoxon signed rank test will also be provided for descriptive purpose among subjects who have received 2 doses of product manufactured by 2 different processes. Similar analysis will be repeated for subjects receiving 2 Flurpiridaz (^{18}F) Injection doses synthesized using the same processes, HPLC or SPE.

The PET-MPI reads will be performed by the same set of readers. In each session, PET images will be displayed in a randomized order, non-sequentially, with PET-MPI exams corresponding to individual subjects randomly allotted into reading session batches. The intra-reader agreement of the detection of ischemic defect on PET-MPI will be assessed by percent agreement and Cohen's kappa value stratified by the 3 groups as used in the primary analysis.

Perfusion and gated acquisitions of rest images will be rated for image quality (excellent, good, fair, poor or non-diagnostic). The image quality score will be summarized descriptively for the 3 groups of data sets (by reader) and the intra-reader agreement will be assessed between the 2 acquisitions stratified by the 3 groups as used in the primary analysis.

The perfusion image quality and gated image quality are categorized into 5 categories (excellent (4), good (3), fair (2), poor (1) and uninterpretable (0)). The image quality agreement for 2 doses from different processes can be defined as follows:

1. SPE image is worse than HPLC image when the difference (SPE – HPLC) score is ≤-3.
2. SPE image is slightly worse than HPTC image when the difference score is ≤-1 and ≥-3.
3. SPE and HPLC images are the same when the difference score is >-1 and <1,
4. SPE image is slightly better when the difference score ≥1 and <3.
5. SPE image is better when the difference is ≥3.

The image quality agreement for 2 doses from the same process may be defined as follows:

1. 2 images are the same when the absolute difference score is ≥0 and <1
2. 2 images are slightly different when the absolute difference score >=1 and ≤3
3. 2 images are different when the absolute difference score >3

The image quality agreement will be summarized with the above-mentioned 5 categories and 3 categories.

Within left ventricle, myocardium, lungs and liver regions of interest (ROIs), a TAC (5-15) (in SUV units) will be generated for the dynamic scan during the 15-minute acquisition period. The SUV for each ROI will be generated using the following formula:

SUV= Decay Corrected Uptake (kBq/cc) / (Injected Dose (MBq) / Weight (kg))

There will be a descriptive statistical analysis only. Bland-Altman method will be used with the relative difference D in the mean SUV expressed as D = 100 (SUV1-SUV2)/[0.5 * (SUV1 + SUV2)] between the 2 processes will be summarized descriptively for each region among subjects who have received 2 doses of product. The mean absolute percentage difference (MAPD) will be determined as the mean of the absolute value of D over all subjects. In addition, the Bland-Altman plots will be presented as well.

6.6 Safety Variables and Analyses

Safety Endpoints: Descriptive summary statistics will be reported for AEs, TEAEs, and SAEs, changes from baseline for clinical laboratory tests, ECGs, physical examination, and vital signs for all treated subjects.

Post-administration changes from baseline will be summarised by mean, SD, minimum, and maximum at each time point. The presentation of all summary data will be presented in 2 manufacturing processes.

6.6.1 Analysis of Adverse Events

AEs and SAEs will be coded using a current version of Medical Dictionary for Regulatory Activities (MedDRA) and all reported events will be listed for the safety population. The version of MedDRA will be detailed in the footnotes of related tables, figures and listings. TEAEs are defined as AEs that occur from the time of IMP administration through the end of the follow-up period. The number and percentage of subjects with 1 or more TEAEs will be summarized by system organ class and preferred term. Summaries and listings will also be presented by AE intensity and judged relationship to IMP. Treatment-emergent SAEs are defined as SAEs that occur from the time of IMP administration through the end of the follow-up period. Treatment-emergent SAEs will also be presented for the safety population.

An AE is defined as any untoward medical occurrence or an already present event that worsens in intensity. A TEAE for Flurpiridaz (^{18}F) Injection is defined as an AE that starts on or after the time of the first injection of Flurpiridaz (^{18}F) Injection until the last safety follow-up visit. The TEAE does not necessarily have to have a causal relationship with exposure to the investigational agent. A TEAE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the exposure to the IMP, whether or not considered related to that product.

The number and percentage of subjects reporting TEAEs will be tabulated by system organ class (SOC) and preferred term (PT). If more than one event occurs with the same PT for the same subject, the subject will be counted only once for that PT using the most severe and most related occurrence for the summarization by severity and by relationship to Flurpiridaz (^{18}F) Injection.

6.6.2 Clinical Laboratory Evaluation

Descriptive statistics will be displayed for the observed values and changes from baseline for the clinical laboratory parameters that contain continuous values shown in Table 4. For the categorical urinalysis parameters, the shift analysis from baseline to post-baseline will be provided. The baseline value for each Period will be defined as the last non-missing value prior to the Period 1 dose or the Period 2 dose. In addition, for each clinical laboratory variable and each time point, the following safety endpoints will be summarised by counts and percentages:

- The occurrence of 1 or more changes from baseline, at each post-administration time point, greater than 40% and 80% of the span of the normal limits (not applicable to qualitative parameters).

- The occurrence of post-administration values outside the normal limits (not applicable to qualitative parameters). Shift tables based on the normal range will be prepared.

Table 4 Clinical Laboratory Parameters

Serum Biochemistry	Haematology	Urinalysis
Alanine aminotransferase (ALT)	Haematocrit	Bilirubin
Albumin	Platelet count	Glucose
Aspartate aminotransferase (AST)	White blood cell (WBC) count	Ketone
Bicarbonate		Occult blood
Bilirubin (total)		pH
Calcium		Protein
Chloride		Urobilinogen
Creatinine		
Gamma-glutamyltransferase		
Glucose		
Lactate dehydrogenase		
Potassium		
Protein (total)		
Sodium		
Urea nitrogen		

6.6.3 Vital Signs

Descriptive statistics will be displayed for the observed values and changes from baseline. For each vital-sign variable and each time point, the following safety endpoints will be summarized by counts and percentages and by manufacturing process:

- The occurrence of 1 or more changes from baseline, at each post-administration time point, greater than a pre-specified magnitude (20 mm Hg for systolic blood pressure, 10 mm Hg for diastolic blood pressure, 10 beats per minute for heart rate, 10 breaths per minute for respiration rate).

- The occurrence of post-administration values outside the normal limits (Table 5). Shift tables based on the normal range will be prepared.

Table 5 Criteria for Normal Limits for Vital Signs

	Normal Limits	
Vital Signs Parameter	**Low**	**High**
Systolic blood pressure, mm Hg	85	139
Diastolic blood pressure, mm Hg	60	89
Heart rate, beats/min	60	100
Respiration rate, breaths/min	12	22
Oxygen saturation, %	93	100
Body weight, kg [a]	41	113
Body mass index (BMI), kg/m^2 [b]	18.5	24.9

[a] Changes in body weight are evaluated by the investigator (without taking height into account), since BMI is not collected on the CRF.

[b] BMI is calculated and analysed retrospectively by the Sponsor, at which time height is taken into account.

6.6.4 Electrocardiograms

Descriptive statistics will be displayed for the observed values and changes from baseline. For each ECG variable and each time-point, the following safety endpoints will be summarized by counts and percentages and by manufacturing process:

- The occurrence of post-administration values outside the normal limits in the PR, QTc, QRS or RR interval (Table 6). Shift tables based on the normal range will be prepared.

ECGs for which the overall interpretation was abnormal will be summarized by counts and percentages at each post-administration time point or by additional/other characteristics deemed necessary by study team.

QTc correction:
The following correction formula will be employed in analyzing QTc data in an attempt to reduce the bias resulting from over- or under-correction:

Fridericia's: $QTcF = QT/\sqrt[3]{RR}$

The following analyses will employ both methods of correction:

- Changes from baseline in the QTc interval will be displayed according to Committee for Medicinal Products for Human Use criteria (absolute QTc interval prolongation, of >450, >480, <500 ms and change from baseline in QTc interval >30 and >60 ms).)

- The number and percentage of subjects with absolute QTc values above the upper limit of normal (Table 6) will be provided. Shift tables based on the normal range will be prepared.

Table 6 Criteria for Normal Limits for ECGs

	Normal Limits (ms)	
ECG Variable	**Low**	**High**
PR interval	120	200
QRS interval	50	100
RR interval	600	1000
QT interval (sex not specified)	—	≤440
QTc interval[a] (sex not specified)	—	≤440

[a] No lower boundary set for QTc.

6.6.5 Physical Examinations

The number and percentage of subjects with changes in physical examination status from normal at pre-administration to abnormal at 1-hour post-administration (and vice versa) will be presented by manufacturing process. Shift tables based on the normal range will be prepared.

6.6.6 Injection Site Monitoring

The findings of injection site monitoring will be summarized by time point. All monitoring information, including date/time and the reasons for “not done” will be listed in a listing.

7 REFERENCES

[Berman et al. 2013]
Berman DS, Maddahi J, Tamarappoo BK, Czernin J, Taillefer R, Udelson JE, et al. Phase II safety and clinical comparison with single-photon emission computed tomography myocardial perfusion imaging for detection of coronary artery disease: flurpiridaz F 18 positron emission tomography. J Am Coll Cardiol. 2013;61(4):469-477.

[Pini et al. 2022]
Pini A, Markström JL, Schelin L. Test–retest reliability measures for curve data: an overview with recommendations and supplementary code, Sports Biomech. 2022;21(2):179-200.

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