Statistical Analysis Plan

Version 2.0 (24JULY2025)

Protocol Title: Intra-patient Comparison of Urinary Radioactivity Following Piflufolastat (18F) and Flotufolastat (18F) PET in Men with Low PSA Biochemical Recurrence of Prostate Cancer Following Radical Prostatectomy

Protocol Number: BED-PSMA-411

Compound Number: Flotufolastat (18F) injection (Trade name: POSLUMA®)

Short Title: N/A

Sponsor Name: Blue Earth Diagnostics Ltd.

Legal Registered Address:

[redacted]

Regulatory Agency Identifier Number(s):

Registry	ID
IND	141561

Table of Contents

List of Tables

Version History

This Statistical Analysis Plan (SAP) for Study BED-PSMA-411 is based on the protocol version 4 dated 22APR2024.

SAP Version	***Date***	***Change***	***Rationale***
1.0	09OCT2024	Not applicable	Original version
2.0	24JUL2025	1. Text for blinding updated to algin with imaging review charter to specify that central readers will be blinded to each radiopharmaceutical 2. Additional PSA group added 3. Added detail in Section 6.3.1.1 that for quantitative analyses of paired organs the left and right values would be averaged and summarised. 4. Major deviations that were agreed during PD review to have potentially affected the evaluation of the PET scan(s) will also be summarised 5. Efficacy detection rate tables/figures repeated also for efficacy analysis set 6. Imputation added for time since event variables (see Section 2.4.3) 7. Detection rate summary by the local site reader in Section 8.2 and demographics and baseline table in Section 12.2 will be repeated for the efficacy analysis set.	1. Updated to align with imaging review charter 2. Additional analysis 3. Clarification *4.* Clarification *5.* Additional analysis *6.* Update due to partial dates *7.* Additional analysis

Signature Page

BED-PSMA-411 SAP Version 2.0 (24JUL2025)

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List of Abbreviations

AE	Adverse Event
ATC	Anatomical Therapeutic Chemical
BCR	Biochemical Recurrence
BMI	Body Mass index
CI	Confidence Interval
CTCAE	Common Terminology Criteria for Adverse Events
DBL	Database Lock
DP	Decimal Place
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
H_0	Null Hypothesis
H_1	Alternative Hypothesis
MAR	Missing at Random
MedDRA	Medical Dictionary for Regulatory Activities
n	Number of Observations
PD	Protocol Deviations
PET	Positron Emission Tomography
PLN	Pelvic Lymph Node
PSA	Prostate-Specific Antigen
PT	Preferred Term
Q-Q	Quantile-Quantile
RP	Radical Prostatectomy
SAP	Statistical Analysis Plan
SOC	System Organ Class
STD	Standard Deviation
SUV	Standardized uptake value
SUV_{max}	Maximum SUV
SUV_{mean}	Mean SUV
SUV_{peak}	Peak SUV
TEAE	Treatment-Emergent Adverse Events
TESAE	Treatment-Emergent Serious Adverse Events
TFL	Table, Figure, and Listing
TNM	Tumor Node Metastasis
USPI	United States Prescribing Information
VOI	Volumes of Interest
WHO-DD	World Health Organisation Drug Dictionary

1. Introduction

This SAP was created using the study protocol version 4 (22APR2025) and electronic case report form (eCRF) version 8.3 (04JUNE2025). A final version of this SAP will be signed off prior to database lock (DBL). Table, Figure, and Listing (TFL) specifications are contained in a separate document.

The SAP analyses supersede those in the protocol. Changes to the protocol-planned analyses, if any, are described in Section 10. Any changes or deviations from this SAP relative to the final analysis will also be fully documented in the clinical study report.

1.1. Objectives and Endpoints

Table 1 below describes the objectives and endpoints, as described in the protocol.

Table 1 Objective and Endpoints

Objectives	**Endpoints**
Primary	
• To compare urinary bladder radioactivity observed on piflufolastat (18F) positron emission tomography (PET) and flotufolastat (18F) PET.	• The difference in urinary bladder mean standardized uptake value (SUV_{mean}) between piflufolastat (18F) and flotufolastat (18F).
Secondary	
• To assess the detection rates for piflufolastat (18F) PET and flotufolastat (18F) PET. • To assess the detection rates stratified by prostate-specific antigen (PSA) level for piflufolastat (18F) PET and flotufolastat (18F) PET. • To assess the prostate bed detection rates: local recurrences by subregion for piflufolastat (18F) PET and flotufolastat (18F) PET. • To assess the pelvic lymph node (PLN) detection rates for piflufolastat (18F) PET and flotufolastat (18F) PET.	• Patient-level detection rate. • Patient-level detection rate by baseline PSA categories. • Detection rate for local recurrences in prostate bed for the following subregions: o Vesicourethral anastomosis lesions. o Retrovesical lesions. o Remnant seminal vesicles/lateral surgical margin lesions. • Detection rate for PLN lesions.
Tertiary	
• To assess Ureteric radioactivity for piflufolastat (18F) PET and flotufolastat (18F) PET.	• Central reader qualitative assessment of the presence or absence of ureteric radioactivity.

• To assess bladder radioactivity for piflufolastat (18F) PET and flotufolastat (18F) PET. • To assess physiological radioactivity for piflufolastat (18F) PET and flotufolastat (18F) PET. • To assess extra-pelvic detection rates for piflufolastat (18F) PET and flotufolastat (18F) PET. • To assess qualitative analysis of urinary radioactivity interference with image assessment for piflufolastat (18F) PET and flotufolastat (18F) PET.	• Maximum SUV (SUV_{max}), peak SUV (SUV_{peak}) and total bladder radioactivity. • SUV_{mean}, SUV_{peak} for normal organs, including liver and spleen. • Detection rate for distant metastatic lesions and in the following subregions: o Bone lesions. o Extra-pelvic lymph node lesions. o Extra-pelvic soft tissue lesions. • Qualitative 3-point scale by expert readers (as previously described by Kuo (Kuo, et al., 2024)).
Safety	
• To assess Treatment-Emergent Adverse Events (TEAEs) after each PSMA PET scan.	• Incidence of TEAEs after each PSMA PET scan.

1.2. Study Design

This is a multi-center, prospective intra-patient comparator study of urinary radioactivity SUV following both piflufolastat (18F) and flotufolastat (18F) PET in patients with low PSA (≤0.5 ng/mL) biochemical recurrence (BCR) of prostate cancer following radical prostatectomy (RP). Approximately 60 patients will be screened for inclusion into the study to obtain a minimum of 52 evaluable patients (i.e. have evaluable PET scans for both piflufolastat (18F) and flotufolastat (18F) available for analysis). As part of their routine clinical care, each patient will be administered a single dose of piflufolastat (18F) on Day 1, followed by a PET scan per standard institutional practice and in line with the PYLARIFY United States Prescribing Information (USPI) (PYLARIFY® [piflufolastat F 18] injection USPI, 2021.). At least 24 hours after the piflufolastat (18F) scan, but within 10 calendar days, all patients will be administered a single dose of flotufolastat (18F) followed by a PET scan. Each patient will receive both radiopharmaceuticals and, thus, serve as their own control.

For the primary endpoint analysis, volumes of interest (VOIs) will be placed over the urinary bladder and radioactivity (SUV metrics) will be assessed following administration of each radiopharmaceutical.

For secondary and tertiary endpoints, both the piflufolastat (18F) and flotufolastat (18F) PET scans will be read by two independent nuclear medicine physicians or nuclear radiologists who are blinded to the patient's clinical history but have been trained to read scans for each respective radiopharmaceutical using product-specific training and in line with the respective FDA-approved USPI for the respective radiopharmaceutical. To facilitate use of the appropriate product-specific training, the independent

readers will be unblinded to the administered agent. Any image interpretation disagreements will be resolved by a third independent adjudicator. Full details are in the Study Image Review Charter.

1.2.1. Blinding

The local sites, including the investigator, will not be blinded as each patient will receive piflufolastat (18F) first as part of their routine clinical care (Day 1), followed at least 24 hours later, but within 10 calendar days, by flotufolastat (18F). For imaging interpretation, central readers will be blinded to the radiopharmaceutical and all other clinical data.

1.2.2. Primary Analysis

The primary analysis will take place after the last patient has had their last visit, all blinded independent evaluation of the images has been performed and the data is finalized, and clinical DBL has been performed.

2. General Considerations

2.1. Statistical Hypotheses

The null hypothesis (H_0) for the primary endpoint is that there is no difference in urinary bladder SUV_{mean} between piflufolastat (18F) and flotufolastat (18F), while the alternative hypothesis (H_1) is that a difference in urinary bladder SUV_{mean} does exist.

$$H_0: Difference\ in\ urinary\ bladder\ SUV_{mean} = 0$$

$$H_1: Difference\ in\ urinary\ bladder\ SUV_{mean} \neq 0$$

2.2. Multiplicity Adjustment

No multiplicity adjustments will be made for this study.

2.3. Impact of Intercurrent Events

Intercurrent events in this study are defined as any event occurring after administration of piflufolastat (18F) that could potentially interfere with the assessment of the primary and secondary endpoints. These events include but are not limited to the following.

2.3.1. Major Protocol Deviations Affecting Evaluation of PET Scans

Major protocol deviations (PDs) that could potentially affect the evaluation of either the piflufolastat or flotufolastat PET scan will be considered as intercurrent events. These events will be assigned during protocol deviation review, and finalized prior to DBL.

The following adjustments will be made in the primary analysis:

- No adjustment. All data (where applicable) will be included irrespective of the occurrence of the intercurrent event.

The following adjustments will be made as a sensitivity analysis:

- Sensitivity Analysis 1: Patients with this intercurrent event will be excluded from the analysis.

2.3.2. Patient Withdrawal After Piflufolastat

In the unlikely event that patients withdraw from the study after the piflufolastat PET scan but before flotufolastat scan, and therefore would have missing data for the flotufolastat scan, the patient withdrawal will be considered as an intercurrent event.

The following adjustments will be made in the primary analysis:

- Patients with only the piflufolastat PET scan will be excluded.

The following adjustments will be made as sensitivity analyses:

- Sensitivity Analysis 2: We will assume the data from the missing flotufolastat PET scan is the same as the piflufolastat PET scan.
- Sensitivity Analysis 3: We will assume the data from the missing flotufolastat PET scan is missing at random (MAR). A mixed model will be used and will include all available patient data.

2.4. Handling of Missing Data

2.4.1. Imputation of Partial Dates for Adverse Events and Concomitant Medications

No imputation of the start and end dates will be performed.

2.4.2. Attributable and Concomitant for Adverse Events and Medications

The following rules will be used to assign attributable to piflufolastat or flotufolastat for adverse events (AEs) and concomitant to piflufolastat or flotufolastat for medications in relation to partial start dates:

- If completely missing or if year is missing, then assume attributable/concomitant to both piflufolastat and flotufolastat.
- If year is present and month and day are missing, then:
    - If year is the same as administration of piflufolastat, then assume attributable and concomitant to piflufolastat.
    - If year is the same as administration of flotufolastat, then assume attributable and concomitant to flotufolastat.
- If year and month are present and day is missing, then:
    - If year and month are the same as administration of piflufolastat, then assume attributable and concomitant to piflufolastat.
    - If year and month are the same as administration of flotufolastat, then assume attributable and concomitant to flotufolastat.

2.4.3. Imputation of Partial Dates for Time Since an Event

For time since an event (see Section 2.7) the following rules will be followed for imputing partial dates:

1. Dates with missing month will have the month imputed to January
2. Dates with missing day will have the day imputed to the first of the month

2.4.4. Causality and Severity

For AEs, if causality is missing then the AE will be assumed to be related. If the severity is missing, the AE will be assumed to be Grade 3.

2.5. Baseline

Baseline for a given variable will be defined as the last non-missing measurement prior to the administration of piflufolastat (18F).

2.6. Study Day

For all summaries, study day is defined in respect to the administration of piflufolastat (18F). For visits or events that occur on or after the administration of piflufolastat (18F), study day is defined as:

$$Date\ of\ visit\ or\ event - \ Date\ of\ piflufolastat\ (18F)\ administration + 1$$

For visits or events that occur prior to the administration of piflufolastat (18F), study day is defined as:

$$Date\ of\ visit\ or\ event - \ Date\ of\ piflufolastat\ (18F)\ administration$$

2.7. Time Since Event

Time since an event in months will be defined as:

$$(Date\ of\ event - \ date\ of\ informed\ consent\ + 1)\ X\ (12/365.25)$$

Events include:

- Initial diagnosis
- Radical prostatectomy
- Biochemical recurrence

2.8. Prior and Concomitant Medications

Medications will be classified as being prior, concomitant to piflufolastat (18F), and/or concomitant to flotufolastat (18F) and are defined as:

- Prior medications are defined as medications with a start date prior to the administration of piflufolastat (18F).
- Concomitant to piflufolastat (18F) medications are defined as medications that are ongoing at the administration of piflufolastat (18F) or have a start date after the administration of piflufolastat (18F).
- Concomitant to flotufolastat (18F) medications are defined as medications that are ongoing at the administration of flotufolastat (18F) or have a start date after the administration of flotufolastat (18F).

Medications may be included in multiple classifications.

2.9. Age

Age at informed consent in years will be derived based on the patient's year of birth:

$$Year\ informed\ consent\ was\ signed - year\ of\ birth$$

2.10. Overall Adjudicated Result

For all detection rate analyses, the detection rates will be calculated separately for each of the blinded readers, and also an overall adjudicated result where disagreements are resolved by a third independent read, allowing adjudication of disagreements by the majority read result.

2.11. General Conventions

Continuous data will be summarized using descriptive statistics for number of observations (n), mean, standard deviation (STD), median, 25th and 75th percentiles, minimum, and maximum. The minimum and maximum will be reported to the same number of decimal places (DPs) as the raw data recorded in the database. The mean, median, lower quartile and upper quartile will be reported to one more DP than the raw data and the STD will be reported to two more DPs than the raw data.

Frequencies and percentages will be used for summarizing categorical data. Percentages will be presented to one DP, apart from 100 which will be presented to 0 DP. Percentages will not be presented for zero counts.

P-values greater than or equal to 0.001 will be presented to three decimal places. P-values less than 0.001 will be presented as "<0.001".

2.12. Listings

Unless specified separately, listings will be created for the following only:

- Discontinued Patients
- PDs
- Patients Excluded from the Efficacy Analysis
- Demographic and Baseline
- Concomitant Medications
- Exposure to Flotufolastat (18F)
- Exposure to Piflufolastat (18F)
- Urinary Bladder SUV_{mean}
- Positive Lesion Detection by Blinded Reader
- AEs; AEs Related to Flotufolastat (18F)
- Serious Adverse Events
- Deaths

2.13. Software

All analyses will be conducted using SAS Version 9.4 or higher.

3. Analysis Sets

For the purposes of analysis, the following analysis sets are defined:

Table 2 Patient Analysis Sets

Patient Analysis Set	Description
All Enrolled Patients	All patients who sign the Informed Consent Form.
Full Analysis Set (FAS)	All patients who receive either piflufolastat (18F) or flotufolastat (18F), and the corresponding PET scan is evaluable.
Full Safety Set (SAF)	All patients who receive either piflufolastat (18F) or flotufolastat (18F), regardless of whether the corresponding PET scan is performed.
Efficacy Analysis Set (EAS)	All patients who receive both piflufolastat (18F) and flotufolastat (18F), and the corresponding PET scans are evaluable.
Per Protocol Set (PPS)	All patients who receive both piflufolastat (18F) and flotufolastat (18F), and the corresponding PET scans are evaluable, and who are without any major PDs that will affect the evaluations of the PET scans.

The FAS, EAS and PPS will be used to analyse endpoints related to the efficacy objectives and the SAF will be used to analyse the endpoints and assessments related to safety.

4. Analyses Supporting Primary Objective

4.1. Primary Endpoint

The primary endpoint for the study is the difference in urinary bladder SUV_{mean} between piflufolastat (18F) and flotufolastat (18F).

4.1.1. Definition of Endpoint

The difference in urinary bladder SUV_{mean} will be calculated for each patient as:

$$Piflufolastat\ (18F)\ SUV_{mean} - Flotufolastat\ (18F)\ SUV_{mean}$$

A positive difference in urinary bladder SUV_{mean} represents a positive result (lower SUV_{mean}) for flotufolastat (18F).

4.1.2. Main Analytical Approach

Summary statistics for urinary bladder SUV_{mean} for piflufolastat (18F), flotufolastat (18F) and their differences will be presented in tables, box and whisker plots, scatter plots (flotufolastat on x-axis and piflufolastat on y-axis) and Bland-Altman plots (difference in urinary bladder SUV_{mean} against their average).

To test the statistical hypothesis specified in Section 2.1, the data will first be checked for normality via. histograms and quantile–quantile (Q-Q) plots. The hypothesis will then be tested using either:

- If the difference in SUV_{mean} is non-normal, a two-sided paired Wilcoxon signed-rank test will be used to test the difference in urinary bladder SUV_{mean} between piflufolastat (18F) and flotufolastat (18F).
- If the difference in SUV_{mean} is normal, a two-sided paired t-test will be used to test the difference in urinary bladder SUV_{mean} between piflufolastat (18F) and flotufolastat (18F).

All analyses will be performed for all patients in the EAS. As all patients in the EAS will have both PET scans evaluable, there will be no missing data in this analysis.

4.1.3. Sensitivity Analysis 1 – Per Protocol Set

To assess the sensitivity of the intercurrent event specified in Section 2.3.1, the first sensitivity analysis for the difference in urinary bladder SUV_{mean} will be done by repeating the primary analysis table on the PPS. This will exclude the patients from the primary analysis who had a major PD that may affect the evaluation of either PET scan.

4.1.4. Sensitivity Analysis 2 – Patient Withdrawal After Piflufolastat [1]

The second sensitivity analysis will assess the sensitivity of the intercurrent event specified in Section 2.3.2. This will be done by repeating the primary analysis on the FAS and imputing missing flotufolastat urinary bladder SUV_{mean} with that patient's piflufolastat urinary bladder SUV_{mean}.

4.1.5. Sensitivity Analysis 3 – Patient Withdrawal After Piflufolastat [2]

The third sensitivity analysis will assess the sensitivity of the intercurrent event specified in Section 2.3.2. This will be done by using a mixed effects linear model fitted to the data for urinary bladder SUV_{mean} between piflufolastat (18F) and flotufolastat (18F). The model will include fixed effect terms for treatment and period, and a random effect for the patient. The least squares means and 95% confidence intervals (CIs) for the treatment groups and estimated differences will be determined and provided, and the FAS will be used. Any missing data will be assumed MAR and maximum likelihood estimation will be used to fit the model parameters.

4.1.6. Supplementary Analysis

The following plots will be created and will include urinary bladder SUV_{mean} among other normal organs:

- Violin plots of SUV_{mean} by normal organs for piflufolastat (18F) and flotufolastat (18F). All organs will be assessed for normality via. histograms and Q-Q plots. For data assessed to be normally distributed the mean with STD error will be presented. For data assessed to be not normally distributed, the median and interquartile range will be presented. This will be summarised for all patients in the EAS.

5. Analyses Supporting Secondary Objectives

5.1. Secondary Endpoint 1

To assess the detection rates for piflufolastat (18F) PET and flotufolastat (18F) PET.

5.1.1. Definition of Secondary Endpoint 1

The patient-level detection rate for piflufolastat (18F) PET and flotufolastat (18F) PET is defined as:

$$\frac{Patients\ with\ positive\ lesions}{Total\ number\ of\ patients}$$

5.1.2. Analytical Approach for Secondary Endpoint 1

The frequency of positive lesions, detection rates and corresponding 95% exact CI will be calculated for piflufolastat (18F) PET and flotufolastat (18F) PET for each blinded reader, and the overall adjudicated result (see Section 2.10). For each blinded reader, the number of positive reads that were adjudicated will also be summarised. A shift table comparison of positive and negative lesions will also be presented for each blinded reader, and the overall adjudicated result. The EAS will be used, and the detection rate table will be repeated for the FAS.

5.2. Secondary Endpoint 2

To assess the detection rates stratified by PSA level for piflufolastat (18F) PET and flotufolastat (18F) PET.

5.2.1. Definition of Secondary Endpoint 2

The first grouping for the PSA stratification will be as follows:

- PSA $\leq$ 0.2
- PSA > 0.2

The second grouping for the PSA stratification will be as follows:

- PSA $\leq$ 0.2
- 0.2 < PSA $\leq$ 0.3
- 0.3 < PSA $\leq$ 0.4
- 0.4 < PSA $\leq$ 0.5

Any PSA subgroup with fewer than 5 patients will be grouped with the adjacent PSA subgroup with the smallest number of patients. If a PSA value is reported as "< X", i.e. below the lower limit of quantification, the value will be converted to X for summaries, but will be presented as recorded, i.e. as "< X" in the listings.

See Section 5.1.1 for the definition of detection rate.

5.2.2. Analytical Approach for Secondary Endpoint 2

The analysis for the patient-level detection rates stratified by PSA level will be performed using the same approach as the patient-level detection rates specified in Section 5.1.2. In addition, bar charts of patient-level detection rates by each PSA group will also be presented.

5.3. Secondary Endpoint 3

To assess the detection rates for local recurrences in the prostate bed for piflufolastat (18F) PET and flotufolastat (18F) PET for the following subregions:

- Vesicourethral anastomosis lesions.
- Retrovesical lesions.
- Remnant seminal vesicles/lateral surgical margin lesions.

If either side (left or right) of the remnant seminal vesicles/lateral surgical margin is positive, then remnant seminal vesicles/lateral surgical margin lesions will be classed as positive.

5.3.1. Definition of Secondary Endpoint 3

See Section 5.1.1 for the definition of detection rate.

5.3.2. Analytical Approach for Secondary Endpoint 3

The analysis for the detection rates for local recurrences by subregion will be performed using the same approach as the patient-level detection rates specified in Section 5.1.2.

5.4. Secondary Endpoint 4

To assess the PLN detection rates for piflufolastat (18F) PET and flotufolastat (18F) PET.

5.4.1. Definition of Secondary Endpoint 4

See Section 5.1.1 for the definition of detection rate.

If either side (left or right) of the PLN is positive, then PLN will be classed as positive.

5.4.2. Analytical Approach for Secondary Endpoint 4

The analysis for the detection rates for PLNs will be performed using the same approach as the patient-level detection rates specified in Section 5.1.2.

6. Analyses Supporting Tertiary Objective(s)

6.1. Tertiary Endpoint 1

To assess the central reader qualitative assessment of the presence or absence of ureteric radioactivity in piflufolastat (18F) PET and flotufolastat (18F) PET.

6.1.1.1. Definition of Tertiary Endpoint 1

The qualitative assessment will be assigned by each blinded reader and will be defined as:

- Absent
- Present

6.1.1.2. Analytical Approach for Tertiary Endpoint 1

The number and percentage of patients with the presence or absence of ureteric radioactivity for piflufolastat (18F) PET and flotufolastat (18F) PET will be summarized for each blinded reader, and the overall adjudicated result (see Section 2.10). The FAS will be used.

6.2. Tertiary Endpoint 2

To assess bladder SUV_{max}, bladder SUV_{peak} and total bladder radioactivity for piflufolastat (18F) PET and flotufolastat (18F) PET.

6.2.1.1. Definition of Tertiary Endpoint 2

See Section 4.1.1 for the definition of difference in SUV.

6.2.1.2. Analytical Approach for Tertiary Endpoint 2

The analysis for SUV_{max}, SUV_{peak} and total bladder radioactivity will be performed using the same approach as the primary analysis specified in Section 4.1.1. The supplementary analysis specified in Section 4.1.6 will also be repeated for SUV_{peak}.

6.3. Tertiary Endpoint 3

To assess SUV_{mean} and SUV_{peak} in normal organs for piflufolastat (18F) PET and flotufolastat (18F) PET.

6.3.1.1. Definition of Tertiary Endpoint 3

See Section 4.1.1 for the definition of difference in SUV.

For the kidneys, lacrimal glands, parotid glands and the sub-mandibular glands where biodistribution is measured on the left and right sides, the average will be calculated and summarised.

6.3.1.2. Analytical Approach for Tertiary Endpoint 3

The analysis for SUV_{mean}, SUV_{peak} in normal organs will be performed using the same approach as the primary analysis specified in Section 4.1.1.

6.4. Tertiary Endpoint 4

To assess the detection rates for distant metastatic lesions for piflufolastat (18F) PET and flotufolastat (18F) PET and for the following subregions:

- Bone lesions.
    - Bone lesions (Pelvis).
    - Bone lesions (Spine).
    - Bone lesions (Ribs).
    - Bone lesions (Other).
- Extra-pelvic lymph node lesions.
- Extra-pelvic soft tissue lesions.

For bone lesions, any positive bone subregion would class as positive for bone lesions.

6.4.1.1. Definition of Tertiary Endpoint 4

See Section 5.1.1 for the definition of detection rate.

6.4.1.2. Analytical Approach for Tertiary Endpoint 4

The analysis for the detection rates for distant metastatic lesions and by subregion will be performed using the same approach as the patient-level detection rates specified in Section 5.1.2.

6.5. Tertiary Endpoint 5

To assess the qualitative 3-point scale by expert readers for piflufolastat (18F) PET and flotufolastat (18F) PET.

6.5.1.1. Definition of Tertiary Endpoint 5

Blinded readers will qualitatively assess the impact of any urinary activity in the bladder on image interpretation using a three-point scale:

0 = No/minimal visible urinary activity.

1 = Urinary activity visible but distinction between urine and disease possible.

2 = Assessment inhibited by urinary activity.

6.5.1.2. Analytical Approach for Tertiary Endpoint 5

The frequency and percentage of each score will be calculated for piflufolastat (18F) PET and flotufolastat (18F) PET for each blinded reader, and the overall adjudicated result (see Section 2.10). A separate summary of scores 0 and 1 combined will also be summarised. Shift tables for each reader, and the overall adjudicated result will also be presented.

Patient-level detection rates will also be summarised, stratified by 0 or 1, and 2 for each blinded reader, and the overall adjudicated result.

The FAS will be used.

7. Safety Analyses

Safety analyses include extent of exposure and AEs. All safety analyses will be performed on the SAF.

7.1. Extent of Exposure

A summary of total administered activity will be provided. All activity will be shown in MBq and mCi using the conversion 1 mCi=37 MBq. Exposure will be summarized for piflufolastat (18F) and flotufolastat (18F).

Doses outside the acceptable ranges will also be summarised:

- Acceptable range for Piflufolastat: Total administered activity 296 MBq to 370 MBq (8 to 10 mCi)
- Acceptable range for Flotufolastat: Total administered activity 236.8 MBq to 355.2 MBq (6.4 to 9.6 mCi)

7.2. Adverse Events

A TEAE is defined as an AE that started or worsened after administration of piflufolastat up to the final safety monitoring telephone call after the administration of flotufolastat (18F) (Visit 4). All AE verbatim terms will be coded by system organ class (SOC) and preferred term (PT) using Medical Dictionary for Regulatory Activities (MedDRA) version 26.1 or higher. TEAEs will be summarized overall and categorized by those attributable to piflufolastat and those attributable to flotufolastat, as described in Table 3.

Table 3 Treatment-Emergent Adverse Event Definitions

Time Period	Categorization
AEs occurring from the time of administration of piflufolastat (18F) (Scan 1; Visit 2) to the earliest of either: • One day after administration of piflufolastat (18F) • The time of administration of flotufolastat (18F) (Scan 2; Visit 3). Note: AEs occurring at the time of flotufolastat will not be attributable to piflufolastat (18F).	Attributable to piflufolastat (18F)
AEs occurring from the time of administration of flotufolastat (18F) (Scan 2; Visit 3) to the earliest of either: • One day after administration of flotufolastat (18F) • Final safety monitoring telephone call after the administration of flotufolastat (18F) (Visit 4) AEs that are considered related to flotufolastat (18F) will also be assigned attributable to flotufolastat (18F).	Attributable to flotufolastat (18F)

The tables will include the number and percentage of patients reporting any event of that term and the total number of events reported.

An overview of TEAEs table will be produced and will include the following categories:

- TEAEs
- TEAEs related to flotufolastat (18F)
- TEAEs by Common Terminology Criteria for Adverse Events (CTCAE) grade
    - Individually by maximum grade
    - Any Grade ≥ 3
- Treatment-emergent serious adverse events (TESAEs)
- TESAEs related to flotufolastat (18F)
- TESAEs resulting in death

The following is a list of summary tables that will also be generated:

- TEAEs by SOC and PT
- TEAEs by descending order of frequency of PT
- TEAEs related to flotufolastat (18F) by SOC and PT
- TEAEs by Maximum CTCAE grade, SOC, and PT
- TESAEs by SOC and PT
- TESAEs related to flotufolastat (18F) by SOC and PT
- TESAEs resulting in death by SOC and PT

For each category and overall, patients reporting more than 1 occurrence for a term (SOC or PT) will be counted only once using the most extreme event (most severe for CTCAE tables).

7.3. Additional Safety Assessments

Not applicable.

8. Other Analyses

Not applicable.

8.1. Subgroup Analysis

Not applicable.

8.2. Image Interpretation by the Local Site Reader

Results of image interpretation by the local site reader for both piflufolastat (18F) PET and flotufolastat (18F) PET will be recorded in the eCRF. The following detection rates and corresponding exact CIs will be summarized:

- Patient-level.
- Local recurrences and subregions as specified in Section 5.3.
- PLN.
- Distant metastatic lesions and subregions as specified in Section 6.4.

The analysis will be done for the EAS and the FAS.

9. Interim Analysis

Not applicable.

10. Changes to Protocol-Planned Analyses

Not applicable.

11. Sample Size Determination

Approximately 60 patients will be screened for inclusion into the study to obtain a minimum of 52 evaluable patients (i.e. have evaluable PET scans for both piflufolastat (18F) and flotufolastat (18F) available for analysis).

A sample size of 52 evaluable patients achieves 80% power to detect a mean of paired differences of 10 in SUV_{mean}, with an estimated STD of paired differences of 25 and with a significance level of 0.05 using a two-sided paired Wilcoxon signed-rank test assuming that the actual distribution of paired differences is uniform.

The difference in SUV_{mean} of 10 is based on the difference in the median bladder SUV_{mean} values of 25.4 observed for another renally cleared PSMA PET ligand, 68Ga-PSMA-11 (Kuten, et al., 2020) and 12.5 observed for flotufolastat (18F) (Kuo, et al., 2024).

12. Supporting Documentation

12.1. Patient Disposition

The following summaries will be made for patient disposition:

- The number of patients screened, and screen failed. To be summarised overall and by site for the all enrolled patients analysis set.
- The number of patients in each analysis set, to be summarised for the all enrolled patients analysis set.
- The number of patients who received piflufolastat (18F) PET, flotufolastat (18F) PET and both piflufolastat (18F) PET and flotufolastat (18F) PET. The number of patients with evaluable scans for piflufolastat (18F) PET, flotufolastat (18F) PET and both piflufolastat (18F) PET and flotufolastat (18F) PET. The number of patients who ended the study early, including reasons, and the number of patients who completed the study. To be summarised for the FAS.

12.2. Baseline Characteristics and Demographics

The following categories will be included in the baseline characteristics and demographics table:

- Age (years) at informed consent (see Section 2.9)
    - Continuous
    - Grouped: < 65; ≥ 65
    - Grouped: < 65; 65 to < 75; ≥ 75
- Ethnicity
- Race
- Baseline weight (kg)
- Baseline height (cm)
- Baseline body mass index (BMI) (kg/m^2)
- Baseline PSA (ng/mL)
    - Continuous
    - Grouped: (See Section 5.2.1)

To be summarised for the FAS and EAS.

12.3. Protocol Deviations

PDs will be handled in accordance with Aixial standard operating procedure “CROSOP207”. PDs will be collected in the PD tracker and stored on the Aixial SharePoint. Major protocol deviations, including those that will affect the evaluations of the PET scans, will be agreed, and signed off prior to DBL.

A summary of all minor and major PDs will be summarised by protocol deviation category for the FAS. Major deviations that were agreed during PD review to have potentially affected the evaluation of the PET scan(s) will also be summarised.

Note that PDs will be collected as “Important” and “Non-Important” but will be presented as “Major” and “Minor” respectively.

12.3.1. Major Deviations That Could Affect the Evaluation of the PET Scans

Major deviations that could affect the evaluation of the PET scans include, but are not limited to:

- Administration time of flotufolastat (18F) PET is < 24 hours from admin time of piflufolastat (18F) PET
- Patients receiving a dose of either diagnostic outside the expected range, defined as:
  - Piflufolastat (18F) PET: 296 - 370 MBq
  - Flotufolastat (18F) PET: 236.8 - 355.2 MBq
- Scan quality is non-diagnostic or outside the specification

All deviations that could affect the evaluation of the PET scans will be discussed during the final PD review meeting at the end of the study, and those subjects with PDs that are agreed to have potentially affected the evaluation of the PET scan(s) will be removed from the PPS (see Section 3).

12.4. Medical History

General medical history data will be coded per MedDRA version 26.1 (or higher) and summarised for the FAS.

12.5. Prior and Concomitant Medications

Medications will be coded using World Health Organisation Drug Dictionary (WHO-DD) version 01MAR2024 B3 or higher. The following summaries will be made for prior and concomitant medications:

- Prior medications
- Concomitant to Piflufolastat (18F) medications
- Concomitant to Flotufolastat (18F) medications

See Section 2.8 for the definitions of prior and concomitant. Summaries will be by Anatomical Therapeutic Chemical (ATC) class text and WHO-DD base substance preferred name for the FAS.

12.6. Prior Prostate Cancer Therapies

The following categories will be summarized for prior prostate cancer therapies:

- Therapy type
- Radiation therapy type
- Radionuclide therapy type
- Time since radical prostatectomy (months) (see Section 2.7)

Patients may have more than one type of prior therapy. Prior prostate cancer therapies will be summarised for the FAS.

12.7. Prostate Cancer History

The following categories will be included in the prostate cancer history summary table:

- Time since initial diagnosis (months) (see Section 2.7)
- Time since documented biochemical recurrence (months) (see Section 2.7)

- Clinical Tumour Node Metastasis (TNM) Stage [1]
- Pathological TNM Stage [1]
- Primary Gleason grade, secondary Gleason grade and total Gleason score from biopsy
- Primary Gleason grade, secondary Gleason grade and total Gleason score from surgery
- Gleason Grade Group [2]

[1] TNM staging will be displayed at observed level and combined at levels T2, T3, T4, M0, M1, MX.

[2] Gleason grade group is defined by the International Society of Urological Pathologists, and the categories are as follow:

- Grade Group 1 is defined as Total Gleason score ≤ 6
- Grade Group 2 is defined as Gleason score 3 + 4 = 7
- Grade Group 3 is defined as Gleason score 4 + 3 = 7
- Grade Group 4 is defined as Total Gleason score of 8
- Grade Group 5 is defined as Total Gleason scores 9-10

Gleason Grade group (using surgery or biopsy if no surgery is available) will be summarized.

Prostate cancer history will be summarised for the FAS.

13. References

Kuo, P. H., Hermsen, R., Penny, R. & Postema, E. J., 2024. Quantitative and qualitative assessment of urinary activity of 18F-flotufolastat-PET/CT in patients with prostate cancer: a post hoc analysis of the LIGHTHOUSE and SPOTLIGHT studies.. *Mol Imaging Biol.,* pp. 26(1):53-6.

Kuten, J., Fahoum, I. & Savin, Z., 2020. Head-to-head comparison of 68Ga-PSMA-11 with 18F-PSMA-1007 PET/CT in staging prostate cancer using histopathology and immunohistochemical analysis as a reference standard.. *J Nucl Med.,* 61(4), pp. 527-32.

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