STATISTICAL ANALYSIS PLAN

Blue Earth Diagnostics

BED-FLC-219

Protocol Title:	An Open-label, Single-arm, Single-dose, Prospective, Multicenter Phase 2b Study to Establish Image Interpretation Criteria for ^{18}F-fluciclovine Positron Emission Tomography (PET) in Detecting Recurrent Brain Metastases After Radiation Therapy (PURSUE)
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1 STATISTICAL ANALYSIS PLAN APPROVAL

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3 LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
COVID-19	Coronavirus Disease of 2019
CSR	Clinical Study Report
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic Case Report Form
FAS	Full Analysis Set
HCG	Human Chorionic Gonadotropin
ICH	International Council for Harmonisation
IEAS	Imaging Evaluable Analysis Set
IMP	Investigational Medicinal Product
LITT	Laser Interstitial Thermal Therapy
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NPV	Negative Predictive Value
PET	Positron Emission Tomography
PPV	Positive Predictive Value
PT	Preferred Term
ROC	Receiver Operating Characteristic
SAE	Serious Adverse Event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SoC	Standard of Care
SOC	System Organ Class
SoT	Standard of Truth
SUV	Standardized Uptake Value
SUV_{max}	Maximum Standardized Uptake Value
SUV_{peak}	Peak Standardized Uptake Value
SUV_{mean}	Mean Standardized Uptake Value
TAC	Time Activity Curve
TBR	Tumor Background Ratio
TBR_{max}	Maximum Tumor to Background Ratio
TBR_{peak}	Peak Tumor to Background Ratio
TEAE	Treatment-Emergent Adverse Event
TESAE	Treatment-Emergent Serious Adverse Event
WHODrug	World Health Organization Drug Dictionary

4 INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to provide comprehensive and detailed descriptions of the methods and presentation of data analyses proposed for Blue Earth Diagnostics Protocol BED-FLC-219 (An Open-label, Single-arm, Single-dose, Prospective, Multicenter Phase 2b Study to Establish Image Interpretation Criteria for ^{18}F-fluciclovine Positron Emission Tomography (PET) in Detecting Recurrent Brain Metastases After Radiation Therapy (PURSUE)). Descriptions of planned analyses are provided in order to avoid post hoc decisions that may affect the interpretation of the statistical analysis. The statistical methods applied in the design and planned analyses of this study are consistent with the International Council for Harmonisation (ICH) guideline *Statistical Principles for Clinical Trials* (E9) (1998).

This SAP will be finalized prior to data analysis to provide full details, including templates for tables, listings, and figures, to be presented in the clinical study report (CSR). Any changes between the statistical methods provided in the clinical study protocol and this SAP will be explained herein; any changes or deviations from this SAP relative to the final analysis will be fully documented in the CSR. Minor changes or deviations from the templates for tables, figures, and listings need not be documented in the CSR.

5 STUDY OBJECTIVES

5.1 Primary Study Objective

The primary objective of this study is

- To establish visual image interpretation criteria for ^{18}F-fluciclovine PET in detecting recurrent brain metastases.

5.2 Secondary Study Objectives

The secondary objectives of this study are:

- To establish other image interpretation criteria for ^{18}F-fluciclovine PET in detecting recurrent brain metastases.
- To assess subject-level diagnostic performance of ^{18}F-fluciclovine PET in detecting recurrent brain metastases.
- To assess lesion-level diagnostic performance of ^{18}F-fluciclovine PET in detecting recurrent brain metastases.
- To assess the safety of ^{18}F-fluciclovine injection in the subject population.

6 INVESTIGATIONAL PLAN

6.1 Overall Study Design

This is a prospective, open label, single arm, single dose study in subjects with solid tumor brain metastases previously treated with radiation therapy, designed to define

image interpretation criteria for ^{18}F-fluciclovine PET in detecting recurrent brain metastases (when read with standard magnetic resonance imaging (MRI) for anatomical reference).

Subjects with a history of brain metastases previously treated with primary, adjuvant or repeat (salvage) radiation therapy, with a recent standard of care (SoC) brain MRI found to be equivocal for recurrent brain metastasis, and who meet all inclusion criteria and none of the exclusion criteria, will be consented and enrolled. Sites are encouraged to enroll subjects with a variety of primary tumor types. In order to ensure variation in tumor types on the study population, the number of subjects with lung cancer will be capped at approximately 60% of enrolled subjects.

On-site investigators will prospectively annotate and measure the 'reference lesion' on a post-radiation treatment MRI scan and on the SoC MRI brain scan to confirm eligibility. The 'reference lesion' is defined as the lesion which is:

- equivocal for recurrent metastasis on SoC MRI (according to inclusion criterion #7, protocol section 6.2);
- intended for pre-planned SoC craniotomy; and
- if >1 equivocal lesion is intended for resection, the largest of these lesions.

If >1 equivocal lesion is intended for resection, the on-site investigator will also annotate and measure the other lesions (termed 'MRI surgical lesions') on the SoC MRI brain scan, defined by the same radiological criteria for a reference lesion per inclusion criterion #7 (see protocol section 6.2).

All eligible subjects will receive an ^{18}F-fluciclovine PET scan (Visit 2 PET) within 42 days of SoC MRI. Subjects will then undergo a repeat study-specific brain MRI scan (Visit 2 On-Study MRI) to be used as anatomical reference for the ^{18}F-fluciclovine PET scan. The Visit 2 On-Study MRI should be done ideally on the same day, otherwise ≤3 days after Visit 2 PET and completed before the pre-planned SoC craniotomy. A safety follow-up (Visit 3) 1 to 3 days after Visit 2 PET will be made for adverse event (AE) evaluation by telephone call or in person (if same day as scheduled SoC craniotomy). AEs occurring from the time of ^{18}F-fluciclovine administration until 1 day post ^{18}F-fluciclovine administration will be recorded. The safety follow-up must be completed before the pre-planned SoC craniotomy. The Visit 2 PET must be organized to take place a minimum of 1 day and maximum of 21 days before pre-planned SoC craniotomy.

The on-site investigator will review the ^{18}F-fluciclovine PET scan to identify, annotate and measure potential additional lesions, not previously reported on SoC MRI (termed 'additional PET lesions'). Additional PET lesions should be annotated only where judged suggestive of brain metastasis, warranting confirmation according to SoC practice, be neurosurgically feasible and accordingly, planned for biopsy or resection. Undertaking biopsy / resection of additional PET lesion(s) identified on the ^{18}F-fluciclovine PET scan is at the discretion of on-site investigators. Moreover, an additional PET lesion should be discrete and spatially separate from known, pre-existing lesions. In particular, the following should not be annotated as Additional PET lesions: known pre-existing non-progressive lesions (considered at site level as

stable or partially responding), ^{18}F-fluciclovine activity contiguous with a known lesion (previously annotated or otherwise) and apparently incongruent with the

lesion's outline on correlating MRI, particularly where this may be reasonably explained by image registration.

For each subject, the final visit will usually be the preplanned SoC craniotomy/surgery at Visit 4. The subject will receive post-procedural management per institutional SoC. All ongoing follow-up and any further treatment will be in accordance with SoC and will not be recorded, including all medications and SoC procedure-related events occurring at and beyond Visit 4, and any SoC follow-up.

Subject-level standard of truth (SoT) will be defined by central histopathological diagnosis of the reference lesion. Lesion-level SoT will be defined by central histopathological diagnosis of all sampled lesions (reference lesions, MRI surgical lesions, additional PET lesions). Separately, central image analysis will define qualitative and quantitative lesion uptake of ^{18}F-fluciclovine, for each resected lesion. These results will be reviewed by an Imaging Interpretation Committee to form image interpretation criteria.

Concurrent Enrollment with BED-FLC-312

Subjects who are enrolled in BED-FLC-312 and during its Follow-Up period are deemed to require a SoC craniotomy for diagnostic evaluation of an equivocal lesion, may be enrolled into BED-FLC-219 while remaining enrolled in BED-FLC-312. Subjects may be enrolled any time during the BED-FLC-312 Follow-Up period after Day 22 (i.e., after the full 21-day maximum timeframe for completion of the BED-FLC-312 Visit 4 neurosurgical procedure). For absolute clarity, this will be irrespective of the applicability of BED-FLC-312 Visit 4 to the subject. Such subjects will be required to sign an informed consent form and meet all eligibility requirements for BED-FLC-219, including availability of the SoC MRI within the specified timeframe and reference lesion definitions. All BED-FLC-219 procedures remain applicable to any such concurrently enrolled subjects. Interim enrollment in BED-FLC-219 does not impact the subject's subsequent ongoing participation in BED-FLC-312.

6.2 Schedule of Assessments

	Day -42 to -1 SoC MRI	Visit 1 Day -28 to -1 Screening	Visit 2 Day 1 IMP + PET	Visit 2 Day 1 to 4 On-Study MRI	Visit 3 Day 2 to 4 Safety Follow-Up Phone Call	Visit 4 Day 2 to 22 SoC Craniotomy
	Before Visit 1 Screening	Within 28 to 1 days before Visit 2 Day 1	1 to 21 days before SoC craniotomy/surgery Visit 4 Day 3	Day of ^{18}F-fluciclovine injection or up to 3 days after	1 to 3 days after ^{18}F-fluciclovine injection, before SoC Craniotomy	At time of pre-planned SoC craniotomy/surgery and after Visit 2 MRI and Visit 3 Follow-Up
SoC MRI	X					
Informed Consent		X				
Confirm inclusion/exclusion criteria		X				
Demographics [a]		X				
Baseline Characteristics [b]			X			
Medical / Disease History [c]		X	X			
ECOG Performance Status [d]		X				
Concomitant Medications		X	X		X	
Vital Signs [e]			X			
Pregnancy Test (Urine) [f]		X	X			
Order ^{18}F-fluciclovine dose		X				
Eligibility Review [g]		X	X			
^{18}F-fluciclovine Injection			X			
^{18}F-fluciclovine PET Brain Scan			X			
Adverse Events			X		X	
On-Study MRI Brain Scan				X		
Annotate Reference Lesion on SoC MRI Brain Scan		X				
Annotate MRI surgical lesions (i.e. other lesions intended for resection per SoC [if applicable])		X				
Identify and annotate additional PET lesions (i.e. potential additional lesions not previously reported on SoC MRI [if applicable]) [h]				X		
Confirm sites of reference lesion and MRI surgical lesions on Visit 2 scans				X		
Safety Follow-up Phone Call					X	
SoC Craniotomy/Surgery						X
Record sites of all sampled lesions						X
Document Neurosurgical Procedure						X
Record local histopathology results and send specimens to central histopathology laboratory						X

ECOG, Eastern Cooperative Oncology Group; IMP, investigational medicinal product; MRI, magnetic resonance imaging; PET, positron emission tomography; SoC, standard of care.

[a] Demographic information recorded at Screening will include age, sex, race and ethnicity.

[b] Baseline assessments will include pre-scan body weight and height.

[c] Treatment history for previous cancer to include previous treatments for brain metastases and previous cancer treatments for primary tumor

[d] A standard of care ECOG assessment may be recorded as the Screening ECOG if the assessment was performed within 28 days of Screening.

[e] Vital signs (body temperature, blood pressure, pulse and respirations) will be collected after resting for at least 5 minutes before IMP injection, and after resting for at least 5 minutes following PET scan.

[f] Females of child-bearing potential.

[g] Eligibility review to include collection and review of prior pre- and post-treatment MRI brain scans as needed to confirm eligibility. Prior pre- and post-treatment MRI brain scans and current SoC MRI images to be uploaded to central imaging core lab.

[h] Additional PET lesions' are potential additional lesions identified by the on-site investigator on ^{18}F-fluciclovine PET scan (with Visit 2 MRI for anatomical reference), not previously reported on SoC MRI, should be judged suggestive of brain metastasis, warranting confirmation according to SoC practice, and be neurosurgically feasible and accordingly, planned for biopsy / resection.

6.3 Treatment(s)

6.3.1 Treatment(s) Administered

Subjects will receive a single dose of ^{18}F-fluciclovine by injection, 185 MBq (5 mCi) ± 20%, delivered as an intravenous bolus by site staff.

6.3.2 Method of Assigning Subjects to Treatment Groups

Not applicable. All subjects will receive a single dose of ^{18}F-fluciclovine.

6.4 Efficacy and Safety Variables

6.4.1 Efficacy Variable(s)

6.4.1.1 Primary Efficacy Variable

The primary efficacy endpoint is:

- Diagnostic performance (sensitivity, specificity, positive predictive value [PPV] and negative predictive value [NPV]) of different thresholds of lesion ^{18}F-fluciclovine uptake on visual reads (as defined by the different combinations of degree of uptake) versus SoT derived from central histopathological analysis of all lesions, at subject-level and lesion-level.

^{18}F-fluciclovine uptake of all sampled lesions, including the reference lesion, will be determined from static imaging data (e.g., from 10-20 minutes post-injection), using the following definitions:

- Absent – ^{18}F-fluciclovine uptake indistinguishable from surrounding structurally normal brain parenchyma
- Mild – ^{18}F-fluciclovine uptake above surrounding structurally normal brain parenchyma, up to or similar to blood pool in the venous sinuses
- Moderate – ^{18}F-fluciclovine uptake higher than blood pool, up to or similar to parotid glands (if parotid gland uptake is asymmetrical, uptake in the gland with higher uptake is used as reference).
- Marked – ^{18}F-fluciclovine uptake higher than parotid glands.

Three thresholds of lesion ^{18}F-fluciclovine uptake, derived from visual reads in the 3 independent reader evaluation, will be considered:

Threshold	**Combination of degree of uptake**
Mild or higher uptake	Absent uptake vs (Mild + Moderate + Marked uptake)
Moderate or higher uptake	(Absent + Mild uptake) vs (Moderate + Marked uptake)
Marked uptake	(Absent + Mild + Moderate uptake) vs Marked uptake

For each threshold, a 2x2 table will be created for each reader at subject level and also at lesion level.

	Tumor Present (SoT)	**Tumor Absent (SoT)**
Threshold Met	*True positive*	*False positive*
Threshold Not Met	*False negative*	*True negative*

Sensitivity, specificity, PPV and NPV for each threshold will be calculated using standard definitions:

- Sensitivity (% of true positives) = true positive / (true positive + false negative)

- Specificity (% of true negatives) = true negative / (true negative + false positive)
- PPV = true positive / (true positive + false positive)
- NPV = true negative / (true negative + false negative)

Subject-level diagnostic performance will be calculated based on reference lesion data, while lesion-level diagnostic performance will be calculated based on data of all sampled lesions.

6.4.1.2 Secondary Efficacy Variables

Secondary efficacy endpoints include the following:

- Diagnostic performance (sensitivity, specificity, PPV and NPV) of different thresholds of quantitative and dynamic measures of lesion ^{18}F-fluciclovine uptake versus SoT derived from central histopathological analysis of all lesions, at subject-level and lesion-level.

- Subject-level sensitivity, specificity, PPV and NPV of ^{18}F-fluciclovine PET scan for detecting recurrent brain metastases, based on combined application of the established image interpretation criteria.

- Lesion-level sensitivity, specificity, PPV and NPV of ^{18}F-fluciclovine PET for detecting recurrent brain metastases, based on combined application of the established image interpretation criteria.

<u>Dynamic measures of lesion ^{18}F-fluciclovine uptake</u>

Time activity curve (TAC) based on ^{18}F-fluciclovine uptake of all sampled lesions, including the reference lesion, on dynamic imaging data will be derived from the following image acquisition frame sequences: first 10 min after injection - 12 frames of 5s, 6 frames of 10s, 6 frames of 30s and 5 frames of 60s; from 10 min to 30 min after injection – 5 minute acquisition frames.

- Type 0: absent uptake
- Type I: continuous increase in uptake throughout the study
- Type II: initial uptake followed by plateau later in the study
- Type III: relatively rapid uptake with reduction in uptake later in the study

For each TAC type, a 2x2 table will be created for subject level and also lesion level, for each reader. Each TAC type will be considered separately (eg. Type 0 vs Type I/II/III). In addition a combination will be evaluated: Type 0/I vs Type II/III.

	Tumor Present (SoT)	**Tumor Absent (SoT)**
TAC Type X	*True positive*	*False positive*
TAC Not Type X	*False negative*	*True negative*

Sensitivity, specificity, PPV and NPV for each TAC type will be calculated in the same way as the primary endpoint.

Quantitative analysis

Lesion(s) and background structures will be outlined visually on static imaging data (e.g., from 10-20 minutes post-injection), and the measures recorded will include:

- lesion maximum standardized uptake value (SUV_{max}),
- lesion peak standardized uptake value (SUV_{peak}),
- background mean standardized uptake value (SUV_{mean}),
- maximum tumor: background ratio (TBR_{max}; tumor SUV_{max}: SUV_{mean} background),
- peak tumor: background ratio (TBR_{peak}; tumor SUV_{peak} : background SUV_{mean}).

6.4.2 Description of Safety Variables

Safety will be assessed from the time of ^{18}F-fluciclovine administration until 1 day post-^{18}F-fluciclovine administration based on reported serious and non-serious adverse events (SAEs and AEs, respectively).

Vital signs, concomitant medications, and pregnancy test results will also be recorded.

6.4.2.1 Adverse Events

An AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or is of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit within the AE collection period. The collection period for all AEs will begin after initiation of ^{18}F-fluciclovine injection and end 1 day post-^{18}F-fluciclovine administration (ie. Day 2). Any unresolved AE at Visit 3 Safety Follow-up beyond Day 2 will be followed until resolution or stabilization. The investigator will record the information in the site's source documents. Adverse events will be recorded in the subject electronic Case Report form (eCRF). Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug.

6.4.2.2 Vital Signs

Vital signs including body temperature, blood pressure, pulse and respirations will be collected at Visit 2 (IMP injection + PET) after resting for at least 5 minutes before IMP injection, and after resting for at least 5 minutes following PET scan. The vital signs should be collected between 5 to 60 minutes before and after the PET scan.

6.4.2.3 Concomitant Medication

All concomitant medication and concurrent therapies will be documented at Screening (Visit 1), at IMP administration (Visit 2) and at the 2-day post-treatment Safety Follow-up phone call (Visit 3), and at early termination when applicable. Dose, route, frequency of administration, and indication for administration and dates of medication will be captured.

6.4.2.4 Laboratory Parameters

A human chorionic gonadotropin (HCG) urine pregnancy test will be obtained from female subjects who are of childbearing age prior to their participation in the study at Screening, and again on Visit 2 before IMP administration (or on day prior to IMP administration).

6.5 Data Quality Assurance

Report summaries will be generated using validated Base SAS® software, version 9.4 or higher, on a PC or server-based platform. Additional validated software may be used to generate analyses, as needed.

All SAS programs that create outputs or supporting analysis datasets will be validated by a second statistical programmer or biostatistician. At a minimum, validation of programs will consist of a review of the program log, review of output or dataset format and structure, and independent confirmatory programming to verify output results or dataset content. Additionally, all outputs will undergo a review by a senior level team member before finalization.

The content of the source data will be reviewed on an ongoing basis by project statistical programmers and statisticians. Data will be checked for missing values, invalid records, and extreme outliers through defensive programming applications, analysis-based edit checks, and other programmatic testing procedures. All findings will be forwarded to the project data manager for appropriate action and resolution.

7 STATISTICAL METHODS

7.1 General Methodology

Data will be analyzed by Precision for Medicine biostatistics personnel. Statistical analyses will be reported with tables, figures, and listings, presented in rich text format, and using recommended ICH numbering. Output specifications for all tables, figures, and listings will be in conformance with guidelines specified by the ICH in Appendix 7 of the *Electronic Common Technical Document Specification* (Apr 2003).

7.1.1 Reporting Conventions

Tables and figures will be summarized for all subjects in the specified analysis set. In general, all data collected and any derived data will be presented in subject data listings, for all enrolled subjects. Listings will be ordered by subject number, and assessment or event date.

In general, continuous variables will be summarized to indicate the study population sample size (N), number of subjects with available data (n), mean, SD, median, minimum, and maximum values. Categorical variables will be summarized by the population size (N), number of subjects with available data (n), number of subjects in each category, and the percentage of subjects in each category. Unless otherwise noted, the denominator to determine the percentage of subjects in each category will be based on the number of subjects in the specified analysis set.

Non-zero percentages will be rounded to one decimal place. Rounding conventions for presentation of summary statistics will be based on the precision of the variable of summarization, as it is collected in its rawest form (i.e., on the eCRF) or as provided within an external file) and are outlined as follows:

- The mean and median will be rounded to one more decimal place than the precision of the variable of summarization;
- Standard deviation will be rounded to two more decimal places than the precision of the variable of summarization; and
- Minimum and maximum values will be presented using the same precision as the variable of summarization.

Other statistics (e.g., CIs) will be presented using the same general rules outlined above, or assessed for the most appropriate presentation based on the underlying data.

7.1.2 Standard Calculations

Where appropriate, the calculated study day of each assessment or event will be presented with the assessment or event date on subject data listings, where study day will be determined as:

- The assessment/event date minus the date of IMP, if the assessment/event date is prior to the date of IMP; and
- The assessment/event date minus the date of IMP, plus one, if the assessment/event date is on or after the date of IMP.

7.2 Analysis Sets

The Full Analysis Set (FAS) will include all enrolled subjects dosed with IMP, with an evaluable Visit 2 on-study PET scan.

The Safety Analysis Set (SAF) will be used for all safety analyses, and will include all subjects who have been dosed with IMP (with or without the associated PET scan).

The Imaging Evaluable Analysis Set (IEAS) includes all subjects who have been dosed with IMP, have an evaluable Visit 2 on-study PET scan, and an evaluable central histopathology report (defined in the Histopathology Charter) from the sample obtained from the Visit 4 SoC craniotomy.

7.3 Study Subjects

7.3.1 Disposition of Subjects

Subject disposition will be summarized for all subjects who entered the study, by study site and overall. Summaries will include the number of subjects enrolled, failed/completed screening, and the number and percentage of subjects in each analysis set, completing the study, and discontinuing the study early by the primary reason for discontinuation. Withdrawals relating to coronavirus disease of 2019 (COVID-19) will be included.

7.3.2 Protocol Deviations

Deviations from the protocol, including those specifically relating to COVID-19, and relevant details will be tracked throughout the study and listed. Critical and major deviations will be summarized for the full analysis set, by study site and overall.

7.3.3 Demographic and Other Baseline Characteristics

Demographic variables including age, sex, ethnicity and race will be summarized for the FAS, SAF and IEAS. Age will be as recorded on the eCRF and will not be re-calculated.

Age will be summarized using descriptive statistics, and age group (<65 years old, ≥65 years old). Sex, ethnicity, and race will be summarized with the number and percentage of subjects in each parameter category.

Baseline characteristics include height, weight, body mass index (BMI), Eastern cooperative oncology group (ECOG) performance status, medical history, and prior treatment history. BMI will be calculated as: weight (kg) / [height (cm) / 100]^2. Height, weight, BMI and ECOG will be summarized for the FAS, SAF, and IEAS; prior treatment history and medical history will be summarized for the FAS only. Height, weight, and BMI at baseline will be summarized using descriptive statistics. ECOG performance status will be summarized with the number and percentage of subjects with each score (0 to 4).

Primary tumor origin and the corresponding details such as disease staging and molecular profiles, where applicable, will be summarized, along with the extra-cranial (systemic) disease status and time since first known date of diagnosis of brain metastasis(es). Primary tumor origin will be categorized as Lung Cancer (non-small cell lung cancer [NSCLC] + small cell lung cancer [SCLC]), NSCLC, SCLC, Breast Cancer, Melanoma, and Other (colorectal, pancreatic, kidney, testes, cervix, ovary, head & neck, osteosarcoma, thyroid, other). Time (months) since first known date of diagnosis will be calculated as (screening date – first known date of diagnosis of brain metastasis(es))/30.4375. If the date of diagnosis is a partial date and day is not available, then day will be imputed as the 15th of the month for the purpose of the calculation. If day and month are both not available then the 1st July will be imputed.

Prior cancer treatments for primary tumor will be summarized with the number and percentage of subjects in each of the following categories:

- Surgery

- Lymph nodes dissection
- Radiotherapy
- Other ablative techniques
- Drug therapy

For the drug therapy category, each drug will be coded using the World Health Organization drug dictionary, WHODrug Global B3 Mar. 1, 2020, grouped into one of the following categories and summarized along with preferred term. The grouped categories will be provided for analysis by a medical expert:

- Chemotherapy
- Immunotherapy
- Targeted Therapy
- Other Therapy

Similarly, concomitant cancer treatments for primary tumor will be summarized by type of treatment (Chemotherapy, Immunotherapy, Targeted Therapy, Other Therapy) and preferred term. The data will come from the Concomitant Medication and Cancer Drug Therapies page, and will include data with the indication for "Treatment for Primary Cancer".

For prior therapies for brain lesions considered as equivocal (i.e. study lesions), the number and percentage of subjects with the following radiotherapies will be summarized:

- Stereotactic radiosurgery
- Whole brain radiotherapy
- Prophylactic cranial irradiation
- Other

The number of lesions (reference lesions and MRI surgical lesions) that have undergone stereotactic radiosurgery will also be counted. The number of subjects and lesions that have undergone laser interstitial thermal therapy (LITT) will be counted.

The number and percentage of subjects with other prior therapies for any non-study lesions, will be summarized using the following categories:

- Craniotomy
- Laser interstitial thermal therapy
- Stereotactic radiosurgery
- Whole brain radiotherapy
- Prophylactic cranial irradiation
- Other

Medical history terms on case report forms will be mapped to preferred terms and system organ classes using the Medical Dictionary for Regulatory Activities

(MedDRA, version 23.0) and will be summarized by system organ class and preferred term. Medical history data will be listed and include start date, end date, ongoing at study start, preferred term and system organ class.

7.4 Efficacy Evaluation

7.4.1 Datasets Analyzed

All efficacy summaries will be based on the IEAS. A data listing of subjects excluded from the IEAS, to include the reason for exclusion, will be presented.

7.4.2 Measurements of Treatment Compliance

Not applicable. This is a single dose study, administered by site staff.

7.4.3 Primary Efficacy Endpoint Analysis Methods

For each uptake threshold (Mild or Higher Uptake, Moderate or Higher Uptake, Marked Uptake), the number and percentage of True Positives, False Positives, True Negatives, and False Negatives will be presented for each reader. Sensitivity, Specificity, PPV and NPV will be calculated, with estimates and the 95% exact confidence intervals displayed.

The summary will be presented for subject-level performance (based on reference lesion data), and repeated for lesion-level performance (based on data of all sampled lesions).

For lesion-level performance, confidence intervals will be calculated based on the methodology described by Zhou et al, to account for clustering within subjects. The formula for PPV is:

$$\widehat{PPV} = \sum_P TP_p \Big/ \sum_P Pos_p$$

$$\widehat{Var}\left(\widehat{PPV}\right) = \frac{1}{P(P-1)} \sum_P \left({Pos_p}/{\overline{Pos}} \right)^2 \left(\widehat{PPV_p} - \widehat{PPV}\right)^2$$

With:

P	: (number of) Patients
p	: individual patient
Pos_p	: Number of positive regions for patient p
TP_p	: Number of true positive regions for patient p
$\overline{Pos} = \sum_P Pos_p / P$	: Mean cluster size
$\widehat{PPV_p} = TP_p / Pos_p$	: Patient PPV

The example in Zhou et al. is coded in SAS in Appendix 1.

In addition, pairwise inter-rater agreements at the lesion level for the 3 readers will be tabulated for the ^{18}F-fluciclovine uptake. The proportions of agreement vs disagreement, Cohen's Kappa and the associated 95% confidence interval will be calculated.

The results of these analyses will be reviewed by the separate Image Interpretation Committee as described in the study protocol to determine image interpretation criteria based on visual reads.

7.4.4 Secondary Endpoint Analysis Methods

In order to establish other image interpretation criteria for ^{18}F-fluciclovine PET in detecting recurrent brain metastases, receiver operating characteristic (ROC) analysis of SoT against the quantitative measures of lesion ^{18}F-fluciclovine uptake will be performed for each reader. A ROC curve will be produced for lesion SUV_{max}, SUV_{peak}, TBR_{max} and TBR_{peak}, with each point corresponding to a specific threshold, eg. $\geq SUV_{max}$ value vs $< SUV_{max}$ value. The AUC of each curve will be calculated using the trapezoidal method and will be annotated on the plot. Sensitivity, specificity, PPV, and NPV will be listed for each value of lesion SUV_{max}, SUV_{peak}, TBR_{max} and TBR_{peak}.

For TBR_{max} and TBR_{peak}, pairwise inter-rater agreement will be assessed using Pearson correlation coefficients.

For dynamic measures of lesion ^{18}F-fluciclovine uptake represented by TAC type, applying each TAC category as a diagnostic criterion, estimates and the 95% exact confidence intervals for sensitivity, specificity, PPV and NPV for each of the 4 TAC types will be calculated and presented, by reader, on both subject and lesion levels. Lesion-level confidence intervals will be based on the method by Zhou et al..

In addition, pairwise inter-reader agreements at the lesion level for the 3 readers will be tabulated for TAC type. The proportions of agreement vs disagreement, Cohen's Kappa and the associated 95% confidence interval will be calculated.

These analyses will be reviewed by the separate Image Interpretation Committee to determine a set of image interpretation criteria based on the quantitative and dynamic measures.

Subsequently, for the diagnostic performance secondary endpoints, estimates and the 95% exact confidence intervals for sensitivity, specificity, PPV and NPV will be calculated based on the recommended thresholds from the following combination of interpretation criteria (if featured in the interpretation criteria):

1. Visual assessment of level of lesion ^{18}F-fluciclovine uptake
2. Strongest performing quantitative measure (based on ROC analysis)

7.4.5 Statistical/Analytical Issues

7.4.5.1 Handling of Dropouts or Missing Data

No imputations will be performed on missing data; all analyses will be based on observed data only.

7.4.5.2 Interim Analyses and Data Monitoring

Not applicable.

7.4.5.3 Multicenter Studies

This is a multicenter study, with approximately 10 sites expected to participate. Data collected from all study sites will be pooled for data analysis.

7.4.5.4 Multiple Comparisons/Multiplicity

There will be no adjustments for multiple comparisons in the efficacy analysis for this study.

7.4.5.5 Examination of Subgroups

The summaries of different thresholds of lesion ^{18}F-fluciclovine uptake, and time activity curve types, on visual reads vs standard or truth, at the lesion-level, will be repeated on the following subgroups:

Primary Tumor Type:

- Melanoma
- Breast
- Lung Cancer (NSCLC and SCLC)
- Gastrointestinal (Colorectal and Pancreatic)
- Genitourinary (Bladder, Kidney, Testes)
- Non-Lung/Breast/Melanoma

Concurrent Immunotherapy:

- Received Concurrent Immunotherapy
- Did Not Receive Concurrent Immunotherapy

Calculations will only be performed when there are at least 10 observations in the subgroup.

7.5 Safety Evaluation

Safety analysis will be carried out for the Safety analysis set, to include all subjects who have been dosed with IMP. Subjects who do not complete the study, for whatever reason, will have all available data up until the time of termination included in the analysis.

7.5.1 Extent of Exposure

Each subject will receive a single dose of ^{18}F-fluciclovine by injection, 185 MBq (5 mCi) ± 20%, delivered as an intravenous bolus by site staff. Total administered activity, in mCi and MBq, will be summarised, along with whether there were any injection site reactions during or immediately after dose, and if a dynamic PET/CT or PET/MRI scan was performed. Other details will be listed.

7.5.2 Adverse Events

Treatment-emergent adverse events (TEAEs) are defined as those AEs with onset after the dose of IMP, or existing events that worsened after the dose of IMP during the study, up to 1 day following IMP administration. Events reported with a partial onset date (e.g., month and year are reported but the day is missing) will be considered to be treatment-emergent if it cannot be confirmed that the event onset was prior to the dose of IMP based on the available date entries.

Adverse event verbatim terms will be coded to preferred terms and system organ classes using the latest version of MedDRA, version 23.0. Severity of AEs will be graded using the national cancer institute common terminology criteria for adverse events (NCI-CTCAE) v5.0.

Summaries that are displayed by system organ class (SOC) and preferred term (PT) will be ordered by descending incidence of SOC and PT within each SOC, and then alphabetically for SOC, and PT within SOC. Summaries of the following types will be presented:

- Overall summary of subject incidence of TEAEs by severity, relationship, and action taken, subject incidence of treatment-emergent serious adverse events (TESAEs) by relationship;
- Subject incidence of TEAEs by MedDRA system organ class and preferred term;
- Subject incidence of TEAEs by NCI-CTCAE grade, MedDRA system organ class, and preferred term;
- Subject incidence of TEAEs related to IMP by MedDRA system organ class, and preferred term;
- Subject incidence of TESAEs by MedDRA system organ class and preferred term.
- Subject incidence of TESAEs related to IMP by MedDRA system organ class and preferred term.

At each level of summarization (e.g., any AE, SOC, and PT), subjects experiencing more than one TEAE will be counted only once. For each subject and each adverse event, the worst severity recorded will be attributed and used in the by-severity summaries.

A by-subject listing of all adverse events (including non-treatment-emergent events) will be provided. This listing will include: subject identifier, age, sex, race, adverse event (SOC, PT, and verbatim term), date of onset and study day of onset, date of resolution and study day of resolution, duration, severity, seriousness, action taken, outcome and causality.

7.5.3 Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

The following listings will be created:

- A by-subject listing of all deaths that occurred during the study, including cause

- A by-subject listing of all serious adverse events

Listings will follow the format described for adverse events in Section 7.5.2.

7.5.4 Clinical Laboratory Evaluation

Urine pregnancy test results will be listed.

7.5.5 Vital Signs

Vital signs recorded values and their respective changes from the pre-dose values will be summarized using descriptive statistics. All results will be listed.

7.5.6 Concomitant Medications

Medications will be coded using WHODrug Global B3 Mar. 1, 2020.

Medications will be considered as prior medications and concomitant medications based on the following rules:

Medication Start date	**Medication Stop date**	**Prior Medication**	**Concomitant Medication**
Prior to date of IMP	Prior to date of IMP	Yes	No
Prior to date of IMP	On or after date of IMP	Yes	Yes
On or after date of IMP	Any	No	Yes

The following imputation rules will be used for determining prior/concomitant medications if the medication start or stop dates are incomplete:

- If medication start and/or stop dates are missing or partial, the dates will be compared as far as possible with the date of IMP. Medications will be assumed to be Concomitant only, unless there is clear evidence (through comparison of partial dates) to suggest that the medication started prior to the dose of IMP.

- If there is clear evidence to suggest that the medication started prior to the dose of IMP, the medication will be assumed to be both Prior and Concomitant, unless there is clear evidence to suggest that the medication stopped prior to the dose of IMP.

- If there is clear evidence to suggest that the medication stopped prior to the dose of IMP, the medication will be assumed to be Prior only.

All prior and concomitant medications will be listed by subject. Concomitant medications will be summarized by anatomical therapeutic chemical (ATC) Class (level 4) and generic drug name, using the safety analysis set. If level 4 is not

available then level 3 will be used; if level 3 and 4 are not available then level 2 will be used.

Concomitant drug therapies relating to the treatment or corticosteroids for brain metastasis / lesions will be summarized separately, in the same way described above. The data will come from the Concomitant Medication and Cancer Drug Therapies eCRF page, and will include data with the indication for "Treatment for Brain Metastasis / Lesion" or "Corticosteroids for Brain Metastasis / Lesion".

7.6 Determination of Sample Size

Up to 40 subjects are planned for enrollment. The sample size is not calculated based on formal statistical hypothesis testing criteria, but is estimated to provide sufficient numbers of subjects and data to establish image interpretation criteria.

7.7 Changes in the Conduct of the Study or Planned Analyses

There were no changes to the study conduct or planned analyses identified within the development of this SAP, relative to the descriptions provided within the clinical study protocol.

8 REFERENCE LIST

Zhou XH, Obuchowski NA and McClish, DK. Statistical Methods in Diagnostic Medicine. Wiley, New York, 2002 pp 104-6

Appendix 1: SAS program to reproduce data in Zhou et al.

```
data one;
input TN      No_Polyps;
Cards;
1      1
2      2
2      2
1      1
2      2
2      2
1      1
1      1
1      1
1      1
2      2
0      1
2      3
2      2
1      1
1      1
1      1
2      2
1      2
0      2
1      1
2      2
2      2
2      2
0      1
;;;;
run;

proc sql;
   create table two as
      select
         TN ,
         No_Polyps,
         TN/No_Polyps as Sei_hat,
         Se_hat,
         No_Polyps/mNo as Ni_N,
         (calculated Ni_N)*(calculated Ni_N)
             * (calculated Sei_hat-Se_hat)* (calculated Sei_hat-Se_hat) as fc,
         sum(calculated fc) as sfc,
         calculated sfc/(n*(n-1)) as varSe_hat
      from
         (select
            sum(TN) as sTN,
            sum(No_Polyps) as sNo,
            mean(No_Polyps) as mNo,
            count(*) as n,
            calculated sTN/calculated SNo as Se_hat
            from one ) as o,
         one;
quit;

data final;
   set two;
   if _n_=1;
   keep Se_hat varSe_hat;
run;
```