STATISTICAL ANALYSIS PLAN

Blue Earth Diagnostics

BED-FLC-312

Protocol Title:	An Open-label, Single-arm, Single-dose, Prospective, Multicenter Phase 3 Study to Establish the Diagnostic Performance of ^{18}F-fluciclovine Positron Emission Tomography (PET) in Detecting Recurrent Brain Metastases after Radiation Therapy (REVELATE)
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Prepared By:	[redacted] Precision for Medicine, Oncology and Rare Disease [redacted]
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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
BIE	Blinded Image Evaluation
BMI	Body Mass Index
COVID-19	Coronavirus Disease of 2019
CSR	Clinical Study Report
CTP	Central Truth Panel
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic Case Report Form
FAS	Full Analysis Set
HCG	Human Chorionic Gonadotropin
ICH	International Council for Harmonisation
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
NPA	Negative Predictive Agreement
NPV	Negative Predictive Value
PET	Positron Emission Tomography
PPA	Positive Predictive Agreement
PPV	Positive Predictive Value
PT	Preferred Term
SAE	Serious Adverse Event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SoC	Standard of Care
SOC	System Organ Class
SoR	Standard of Reference
SoT	Standard of Truth
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-312 (An Open-label, Single-arm, Single-dose, Prospective, Multicenter Phase 3 Study to Establish the Diagnostic Performance of ^{18}F-fluciclovine Positron Emission Tomography (PET) in Detecting Recurrent Brain Metastases after Radiation Therapy (REVELATE)). 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 and listings, 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 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 assess the negative percent agreement (NPA) and positive percent agreement (PPA) of ^{18}F-fluciclovine PET in detecting recurrent brain metastases on a subject-level.

5.2 Secondary Study Objectives

The secondary objectives of this study are:

- To assess other diagnostic performance parameters of ^{18}F-fluciclovine PET in detecting recurrent brain metastases on a subject-level.
- To assess lesion-level diagnostic performance of ^{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 in different clinical settings.
- To evaluate the added clinical usefulness of ^{18}F-fluciclovine PET in evaluation of subjects with suspected recurrent brain metastases.
- To establish inter- and intra-reader reproducibility of ^{18}F-fluciclovine PET image interpretation for 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 establish the diagnostic performance of ^{18}F-fluciclovine PET (read with standard magnetic resonance imaging [MRI] for anatomical reference) in detecting recurrent brain metastases where MRI is equivocal.

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. The study population will be balanced to represent different primary tumor types of any origin, with a minimum of approximately 15% of the cohort each with lung cancer, breast cancer and melanoma. The number of subjects with lung cancer will be capped at approximately 50% of enrolled subjects.

Investigator will prepare a diagnostic management plan prior to the ^{18}F-fluciclovine PET scan and will update the plan following the scan. 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 for anatomical reference, ideally on the same day, and potentially at the same time, as a PET/MRI scan, otherwise ≤3 days after Visit 2 PET and completed before any pre-planned neurosurgical intervention when applicable. A Safety Follow-up (Visit 3) 1 to 3 days after investigational medicinal product (IMP) injection/PET (Visit 2 PET) will be made for adverse event (AE) evaluation by telephone call or in person (if same day as other scheduled SoC appointments). 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 biopsy/neurosurgical intervention, if applicable. Subjects who experience a serious adverse event (SAE) or an AE that persists at Visit 3, will be followed until resolution or stabilization of these events. All ongoing follow-up and any further treatment will be in accordance with SoC.

As MRI is not specific for tumor recurrence in this setting, current clinical practice is mainly focused on close clinical follow up to determine the presence of recurrence. Alternatively, biopsy or direct neurosurgical intervention of the abnormality may be performed. This study will utilize all available diagnostic and follow-up clinical data for up to six months post PET scan, to establish the underlying diagnosis. The planned diagnostic management plan will be recorded at Screening and assessed for any changes following ^{18}F-fluciclovine PET scan.

If biopsy/neurosurgical intervention has been pre-planned per SoC, the Visit 2 PET will be organized to take place a minimum of 1 day and maximum of 21 days before pre-planned SoC biopsy or neurosurgical intervention.

On-site investigators will prospectively annotate and measure the 'reference lesion' on a post-radiation treatment MRI scan and on the pre-study SoC MRI scan to confirm eligibility.

The 'reference lesion" is defined as the lesion which is:

a. Equivocal for recurrent metastasis on SoC MRI (according to inclusion criterion #7, see protocol section 6.2).
b. Intended for SoC biopsy/neurosurgical intervention procedure (if planned). If >1 lesion is intended for biopsy/intervention, the largest of these lesions will be the reference lesion.
c. Where clinical follow-up is planned, the largest of all equivocal lesions (if >1 is present) will be the reference lesion.

If >1 equivocal lesion is under evaluation as SoC, the on-site investigator will also annotate 'other equivocal lesions' on the pre-study SoC MRI scan, defined as follows:

a. Equivocal for recurrent metastasis on SoC MRI (according to the same radiological criteria for a reference lesion per inclusion criterion #7, see protocol section 6.2).
b. Where SoC biopsy/neurosurgical intervention procedure is planned, these are the other equivocal lesions pre-planned for biopsy/intervention.
c. Where SoC clinical follow-up is planned, these are the other equivocal lesions pre-planned for SoC clinical follow-up.

The on-site investigator will also review the ^{18}F-fluciclovine PET scan to identify 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 by clinical follow-up, biopsy or resection, according to SoC practice. 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 subjects due for pre-planned SoC biopsy/neurosurgical intervention, the procedure will be 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. Samples obtained during this Visit 4 will be subject to central histopathological evaluation.

As with subjects who undergo pre-planned SoC biopsy/neurosurgical intervention, subjects pre-planned for clinical follow up to evaluate the equivocal lesions will undergo follow-up performed as SoC per local practice. This is anticipated to vary between subjects.

The final visit for each subject, including subjects who had undergone pre-planned SoC biopsy/neurosurgical intervention, will usually be the Month-6 Follow-up at Visit 5. For study purposes, clinical data accrued during SoC clinical follow-up, pertaining to the ongoing assessment, eventual confirmation of diagnosis, and treatment of the lesions, will be collected. The data will comprise brain imaging scans, treatment regimens, histopathological reports and mortality status. The period of data accrual will be up to 6 months following ^{18}F-fluciclovine PET, or death, whichever comes first. Subjects still considered equivocal at site level at Month 6 (Visit 5) will undergo a study MRI.

With the exception of PET scans, a set of clinical follow-up data (specified in the Central Truth Panel charter) and, where applicable, central histopathology results, will be reviewed by a Central Truth Panel (CTP) to assign a final diagnosis to each lesion, and form the Standard of Reference (SoR). Separately, ^{18}F-fluciclovine PET scans will be centrally read in a blinded image evaluation (BIE), using image interpretation criteria established from the separate Phase 2 study (BED-FLC-219). Diagnostic performance primary and secondary endpoints will then be derived from BIE scan reads matched against SoR. Subject-level SoR will be defined by CTP diagnosis of the reference lesion. Lesion-level SoR will be defined by CTP diagnosis of all lesions (reference lesions, other equivocal lesions, additional PET lesions).

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 Biopsy / Neurosurgical Intervention (if applicable)**	**Visit 5 Month 6 (183 days ± 14 days) Follow-up**
	Before Visit 1 Screening	Within 28 to 1 day before Visit 2 Day 1	1 to 21 days before SoC optional Biopsy / Neurosurgical Intervention 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 optional SoC Biopsy /Neurosurgical Intervention (if applicable)	If applicable, at time of pre-planned SoC Biopsy / Neurosurgical Intervention and after Visit 2 MRI and Visit 3 Follow-Up	Through 6 months after ^{18}F-fluciclovine PET
SoC MRI	X						
Informed Consent		X					
Confirm inclusion/exclusion criteria		X	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				
Diagnostic Management Plan		X	X				
Order ^{18}F-fluciclovine dose		X					
Eligibility Review [g]		X					
^{18}F-fluciclovine Injection			X				
Dynamic PET Brain Scan			X				
Adverse Events			X		X		
On-Study MRI Brain Scan				X			X [h]
Annotate Reference Lesion on SoC MRI Brain Scan		X					
Annotate other equivocal lesions (if applicable) [i]		X					

	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 Biopsy / Neurosurgical Intervention (if applicable)	Visit 5 Month 6 (183 days ± 14 days) Follow-up
	Before Visit 1 Screening	Within 28 to 1 day before Visit 2 Day 1	1 to 21 days before SoC optional Biopsy / Neurosurgical Intervention 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 optional SoC Biopsy /Neurosurgical Intervention (if applicable)	If applicable, at time of pre-planned SoC Biopsy / Neurosurgical Intervention and after Visit 2 MRI and Visit 3 Follow-Up	Through 6 months after ^{18}F-fluciclovine PET
Identify and annotate additional PET lesions (i.e. potential additional lesions not previously reported on SoC MRI [if applicable]) [j]			X				
Safety Follow-up Phone Call					X		
SoC Biopsy/Neurosurgical Intervention						X	
Document Neurosurgical Procedure						X	
Record local histopathology results and send specimens to central histopathology laboratory						X	
Neurological Deterioration Assessment							X
Clinical Follow-Up / Data abstraction [k]							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. Pre-scan body weight and height will be collected at Visit 2.

[c] Treatment history for previous cancer to include previous treatments for brain metastases and previous cancer treatments for primary tumor. Any changes to the subject's medical condition between Screening (Visit 1) and the start of ^{18}F-fluciclovine administration (Visit 2) should be recorded as updated medical history.

[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 childbearing potential.

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

[h] A study MRI will be performed for any subject whose reference or other equivocal lesion(s) is/are still considered equivocal at site level at Visit 5 / Month 6 unless a SoC MRI was performed within the preceding 3 weeks.

[i] If >1 equivocal lesion is under evaluation as SoC, the on-site investigator will also annotate 'other equivocal lesions' on the Visit 2 On-Study MRI brain scan, defined as follows:

a) Equivocal for recurrent metastasis on SoC MRI (according to the same radiological criteria for a reference lesion per inclusion criterion #7).
b) Where SoC biopsy/neurosurgical intervention procedure is planned, these are the other equivocal lesions pre-planned for biopsy/intervention.
c) Where SoC clinical follow-up is planned, these are the other equivocal lesions pre-planned for SoC clinical follow-up.

[j] Additional PET lesions are potential additional lesions identified by the on-site investigator on ^{18}F-fluciclovine PET scan, not previously reported on SoC MRI, and judged to be suggestive of brain metastasis, warranting confirmation by clinical follow-up, biopsy or resection according to SoC.

[k] Relevant clinical and imaging data will be uploaded for up to 6 months following ^{18}F-fluciclovine PET scan.

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:

- Subject-level NPA and PPA (equivalent to specificity and sensitivity respectively) of ^{18}F-fluciclovine PET in detecting recurrent brain metastases.

Subject level NPA and PPA will be calculated based on BIE of ^{18}F-fluciclovine PET compared to SoR on the reference lesion, for each of the 3 readers, as follows:

	SoR - Positive	**SoR - Negative**
Reader X – Positive	*True positive*	*False positive*
Reader X – Negative	*False negative*	*True negative*

- PPA (% of true positives) = true positive / (true positive + false negative)
- NPA (% of true negatives) = true negative / (true negative + false positive)

6.4.1.2 Secondary Efficacy Variables

Secondary efficacy endpoints include the following:

- Subject level positive predictive value (PPV) and negative predictive value (NPV) of ^{18}F-fluciclovine PET for detecting recurrent brain metastases.
  - PPV = true positive / (true positive + false positive)
  - NPV = true negative / (true negative + false negative)
- Lesion-level PPA, NPA, PPV and NPV of ^{18}F-fluciclovine PET for detecting recurrent brain metastases
- Sub-group analyses of subject-level PPA, NPA, PPV and NPV of ^{18}F-fluciclovine PET, according to primary tumor type and concurrent immunotherapy.
- Clinical usefulness:
  - Number of days taken by the site to establish presence/absence of metastasis by clinical follow-up.
  - Proportion of subjects with additional metastases identified on ^{18}F-fluciclovine PET in addition to standard of care brain MRI.
  - Proportion of subjects whose prospective diagnostic management plan changes following ^{18}F-fluciclovine PET.

- Inter-reader and intra-reader agreement statistics (kappa coefficient).
- Treatment-emergent adverse events (TEAEs) following ^{18}F-fluciclovine injection in the subject population

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.

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 (i.e., 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 post-treatment Safety Follow-up phone call (Visit 3), and at early termination when applicable. In addition to recording all concomitant medications as described, treatments and therapies for the brain lesion(s) and primary tumor will be recorded through Month 6 (Visit 5). 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 and listings, presented in rich text format, and using recommended ICH numbering. Output specifications for all tables 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 will be summarized for all subjects in the specified analysis set. In general, all data collected on the eCRF plus data received by Precision for Medicine from the image management system, 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 FAS will be used for primary and secondary non-safety endpoints (including diagnostic performance and inter-reader and intra-reader assessment for the PET scans).

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

7.3 Study Subjects

7.3.1 Disposition of Subjects

Subject disposition will be summarized for all subjects who entered the study. Summaries will include 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.

7.3.3 Demographic and Other Baseline Characteristics

Demographic variables including age, sex, ethnicity and race will be summarized for the FAS and SAF. 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 and SAF; 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).

The number of subjects with confirmed equivocal reference lesions by central neuroradiologist will be summarized.

The prevalence data (% positive reference lesions according to SoR) will be summarized.

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, sarcoma, 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 1st of

the month for the purpose of the calculation. If day and month are both not available then the 1st January 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 other equivocal 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 (including grade), preferred term and system organ class.

7.4 Efficacy Evaluation

7.4.1 Datasets Analyzed

All efficacy summaries will be based on the FAS. A data listing of subjects excluded from the FAS, 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

The primary endpoint for the study is subject-level NPA and PPA (equivalent to specificity and sensitivity, respectively) of ^{18}F-fluciclovine PET in detecting recurrent brain metastases.

Subject-level NPA and PPA will be calculated based on BIE of ^{18}F-fluciclovine PET compared to SoR on the reference lesion. Imputations for SoR indeterminate findings will be performed according to section 7.4.5.1.

The analyses for NPA and PPA will be performed using the one-sample binomial exact test to test the following hypotheses:

<u>NPA</u>

- $H_0(_{NPA})$: NPA of PET $\leq NPA_0$
- $H_1(_{NPA})$: NPA of PET $> NPA_0$

<u>PPA</u>

- $H_0(_{PPA})$: PPA of PET $\leq PPA_0$
- $H_1(_{PPA})$: PPA of PET $> PPA_0$

where NPA_0 and PPA_0 are performance goals for NPA and PPA, respectively. Performance goals of 50% (NPA_0) for NPA and 50% (PPA_0) for PPA have been selected as acceptable thresholds given the setting where SoC imaging is already equivocal.

Exact two-sided 95% CIs according to the Clopper-Pearson method will be provided. If the pre-defined NPA and PPA goals are met by the same two of three readers (both tests have lower limits for the exact 95% confidence intervals greater than 50% for the same two readers), the study will be considered to have successfully demonstrated the effectiveness of ^{18}F-fluciclovine PET in detecting recurrent brain metastases.

7.4.4 Secondary Endpoint Analysis Methods

7.4.4.1 Subject-Level Positive Predictive Value / Negative Predictive Value

Subject-level PPV and NPV of ^{18}F-fluciclovine PET for detecting recurrent brain metastases will be calculated based on BIE of ^{18}F-fluciclovine PET compared to SoR on the reference lesion. The point estimates for PPV and NPV, together with the 95% 2-sided exact confidence interval (Clopper-Pearson method) will be presented.

7.4.4.2 Lesion-Level Diagnostic Performance

Lesion-level diagnostic performance (lesion-level PPA, NPA, PPV and NPV) will be calculated based on BIE of ^{18}F-fluciclovine PET compared to SoR on all lesions (reference, other equivocal and additional PET lesions) as determined by CTP (i.e. if CTP decides that the lesion SoR is indeterminate, then the lesion will not be counted in this lesion-level diagnostic performance). Point estimates and 95% 2-sided confidence intervals will be presented.

The confidence interval will be calculated based on the methodology described by Zhou et al, for lesion-level PPA, NPA, PPV and NPV. 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.

7.4.4.3 Subgroup Analysis of Diagnostic Performance Data

Subgroup analyses of subject-level PPA, NPA, PPV, and NPV of ^{18}F-fluciclovine PET, will also be presented. Subgroups will be as follows, and the calculations will only be performed when there are at least 10 observations in the subgroup:

Age Group:

- <65 years old
- ≥ 65 years old

Sex:

- Male
- Female

Race:

- American Indian or Alaska Native
- Asian
- Black or African American
- Native Hawaiian or Other Pacific Islander
- White

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

7.4.4.4 Clinical Usefulness

7.4.4.4.1 Number of Days taken by the site to establish presence/absence of metastasis by clinical follow-up

The number of days taken by the site to establish presence/absence of metastasis for each lesion, by clinical follow up, will be defined as the time from administration of ^{18}F-fluciclovine to the date the presence or absence of metastasis is determined in each lesion by the site. Analysis will be performed at the lesion-level (including all lesions - reference, other equivocal, additional PET) recorded on the lesion annotation page of the eCRF, and at the subject-level (based on the reference lesion only).

If the presence or absence of metastasis is not ascertained by the time the subject reaches the end of the follow-up or when the subject prematurely withdraws from the study, the lesion/subject will be censored on the date the subject withdraws from the study. The number of diagnoses ascertained and the number censored will be summarized. Kaplan-Meier methodology will be used to handle the censoring of the data, and to estimate the median time to establish presence/absence of metastasis. The range of days taken to establish presence/absence will be presented for all lesions/subjects and also for uncensored lesions/subjects.

7.4.4.4.2 Proportion of subjects with additional metastases identified on ^{18}F-fluciclovine PET in addition to standard of care brain MRI.

Subjects with additional metastases identified on ^{18}F-fluciclovine PET but not on SoC MRI are those with at least one lesion with type = 'Additional PET Lesion' on the lesion annotation eCRF page, which has SoR = 'positive' according to the CTP. Lesions that are deemed to have SoR = 'indeterminate" by CTP will not be considered as additional metastases.

The estimate of the proportion of subjects and the associated 95% 2-sided exact confidence interval (Clopper-Pearson) will be presented.

7.4.4.4.3 Proportion of subjects whose prospective diagnostic management plan changes following ^{18}F-fluciclovine PET.

The proportion of subjects whose prospective diagnostic management plan changes following ^{18}F-fluciclovine PET will be established by comparing the diagnostic management plan of the reference lesion before and after ^{18}F-fluciclovine PET.

The intended diagnostic management plan on the reference lesion will be recorded at visit 1 as craniotomy, biopsy, biopsy in context of LITT, or clinical follow-up. At visit 2, post ^{18}F-flucicolvine PET, the diagnostic plan will be recorded again. If a different option has been chosen, or if there are any additional/new subject management activities planned then this will be counted as a change.

The estimate of the proportion of subjects and the associated 95% 2-sided exact confidence interval (Clopper-Pearson) will be presented.

7.4.4.5 Inter-Reader / Intra-Reader Agreement (Blinded Image Evaluation)

For inter-reader agreement, pairwise comparisons of the ^{18}F-fluciclovine PET scan central reads for the 3 readers (i.e. Reader 1 vs Reader 2, Reader 1 vs Reader 3, and Reader 2 vs Reader 3) will be performed at the subject-level (i.e., reference lesion only) and also at the lesion-level (i.e., all lesions defined by site). The number and percentage of each combination of results (Positive [1st reader] / Positive [2nd reader], Positive/Negative, Negative/Positive, Negative/Negative) will be presented, along with the number and percentage of results in agreement and disagreement. Cohen's kappa statistic will be calculated and presented for each pairwise comparison of the 3 readers, with the corresponding 95% CI and p-value.

Similar statistics will be presented for intra-reader agreement (10% of subjects) – comparisons will be between the initial read vs re-read of a subset of PET scans for each reader.

7.4.4.6 Sensitivity Analyses

7.4.4.6.1 Analysis 1: Subject-Level NPA and PPA on subjects with histopathology or follow-up MRI prior to CNS-directed therapy

Subject-level NPA and PPA (equivalent to specificity and sensitivity, respectively) of ^{18}F-fluciclovine PET in detecting recurrent brain metastases will be calculated for subjects who either have histopathology available, or have follow-up MRI acquired before new or altered CNS-directed therapy and have lesions with at least 20% increase or 30% decrease in the longest diameter relative to the SoC baseline MRI.

The SoR will be based on CTP determination on the two corresponding domains: histopathology and on MRI findings before new or altered CNS-directed therapy.

- SoR will be positive if one domain is positive and the other domain is positive or non-contributory.
- SoR will be negative if one domain is negative and the other domain is negative or non-contributory.
- SoR will be indeterminate if both domains are non-contributory, or if one domain is positive and the other domain is negative.

7.4.4.6.2 Analysis 2: Subject-Level NPA and PPA on subjects without contributory MRI findings after new or altered CNS-directed therapy

Subject-level NPA and PPA (equivalent to specificity and sensitivity, respectively) of ^{18}F-fluciclovine PET in detecting recurrent brain metastases will be calculated for subjects who:

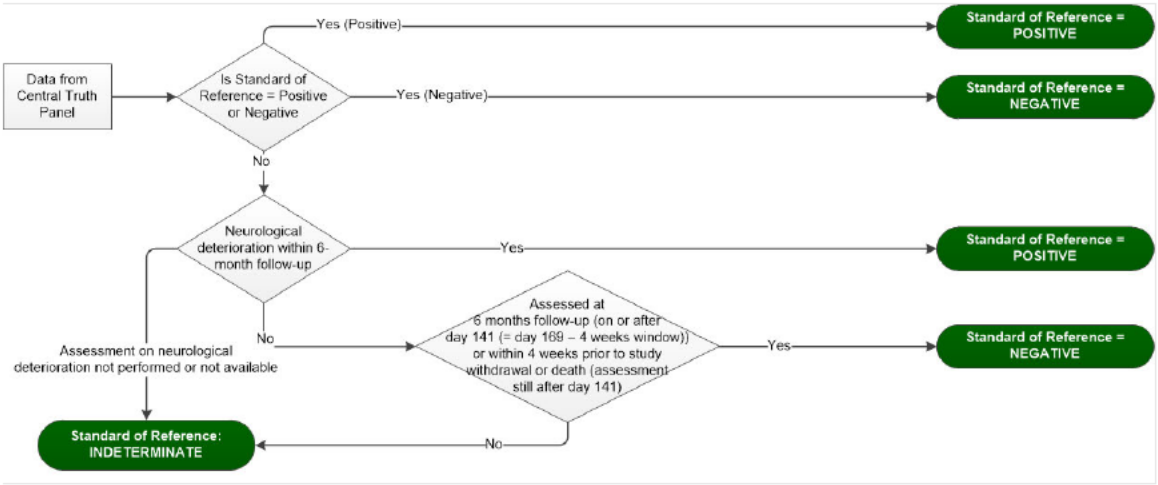
- have no CNS-directed therapy during follow-up, or
- have non-contributory findings in MRIs acquired after new or altered CNS-directed therapy.

The SoR will be based on the final CTP determination in these subjects.

7.4.5 Statistical/Analytical Issues

7.4.5.1 Handling of Dropouts or Missing Data

Data imputation on missing or indeterminate SoR from the Central Truth Panel on the reference lesion (i.e. subject level only) will be performed according to the following chart:



If the SoR determined by the CTP is not positive/negative then the SoR will be determined using the following rules:

1. If the subject has neurological deterioration at any time during the 6-months follow-up, the SoR will be assigned to positive.

2. If the subject is confirmed not to have neurological deterioration on or after day 141 (6 months with a 4-week time window, i.e. day 169 with a 28-day time window), even if the subject dies after day 141, the SoR will be assigned to negative.

3. Otherwise the SoR will remain as indeterminate.

All subject-level analysis involving SoR will be presented twice, by imputing the remaining indeterminate results as follows:

1. Main analysis of primary and secondary endpoints on subject level diagnostic performance – for each PET reader, the worst case according to that reader (i.e. the opposite finding to the individual PET reader finding) will be imputed

2. Sensitivity analysis 1 of primary and secondary endpoints on subject level diagnostic performance - for each PET reader, the worst case according to the majority of PET readers (i.e. the opposite finding to the PET finding of at least 2/3 PET readers)

To check the validity of the results based on the site's determination of the reference equivocal lesions, these lesions will be measured and confirmed by the central neuroradiologist. A sensitivity analysis on the main endpoint of subject level PPA and NPA, using only subjects with central neuroradiologist confirmation of the reference equivocal lesions, will be performed if there is discrepancy between the site confirmation of reference equivocal lesions vs the central neuroradiologist confirmation.

For lesion level analysis, none of the lesions will be imputed if the SoR designation from the CTP is missing or indeterminate. Lesion-level analysis will only include lesions with SoR designation of positive or negative by the CTP.

7.4.5.2 Interim Analyses and Data Monitoring

Not applicable.

7.4.5.3 Multicenter Studies

This is a multicenter study, with approximately 18 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

Subgroup analysis will be performed as described in section 7.4.4.3.

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. IMP administration data will be summarized, and details of administration will also be listed.

7.5.2 Adverse Events

Treatment-emergent adverse events 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 and cause of death that occurred during the study, including the 6-month follow-up
- 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

Approximately 150 subjects will be enrolled, to obtain 130 evaluable subjects.

A one-sample binomial exact test will be used to test the following hypotheses in NPA and PPA of ^{18}F-fluciclovine PET:

NPA

- $H_0(_{NPA})$: NPA of PET $\leq NPA_0$
- $H_1(_{NPA})$: NPA of PET $> NPA_0$

PPA

- $H_0(_{PPA})$: PPA of PET $\leq PPA_0$
- $H_1(_{PPA})$: PPA of PET $> PPA_0$

Where NPA_0 and PPA_0 are performance goals for NPA and PPA, respectively.

Performance goals of 50% (NPA_0) for NPA and 50% (PPA_0) for PPA were selected as acceptable thresholds given the setting where SoC imaging is already equivocal. The lower limits for the exact 95% confidence intervals (Clopper-Pearson method) for both NPA and PPA should be greater than 50%. Assuming a NPA of at least 70% and PPA of at least 70% for ^{18}F-fluciclovine PET would need to be observed, a sample size of 130 would achieve 90% power at a 1-sided significance level of 0.025 to reject the performance goals for both NPA and PPA; assuming a brain metastasis prevalence rate of 0.5.

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;
```