BLUE EARTH DIAGNOSTICS
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CLINICAL STUDY PROTOCOL

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)

BED-FLC-219

Phase: 2b

IND Number: 145265

Protocol Version and Date: Protocol Version 3.0, 10 June 2021

Sponsor: Blue Earth Diagnostics

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Investigational Product:	^{18}F-fluciclovine / Fluciclovine (^{18}F)
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Development Phase:	2b
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Funding Organization:	Blue Earth Diagnostics, Ltd
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Chief Medical Officer
Blue Earth Diagnostics

Date

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ADR	Adverse drug reaction
AE	Adverse event
ALARA	As low as reasonably achievable
ASCT2	Alanine-serine-cysteine-transporter 2
BED	Blue Earth Diagnostics
CBP	Child-bearing potential
CE	Contrast enhancement
CLR	Complete lesion response
CT	Computerized tomography
CTCAE	Common Terminology Criteria for Adverse Events
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report form
EDC	Electronic data capture
EU	European Union
^{18}F	Isotope of fluorine
FACBC	^{18}F-labeled 1-amino-3-fluorocyclobutane-1-carboxylic acid
FAS	Full Analysis Set
FDA	Food and Drug Administration
FDG	Fluorodeoxyglucose
^{18}F-FDOPA	^{18}F-fluoro-dihydroxyphenylalanine
^{18}F-FET	^{18}F-fluoro-ethyltyrosine
FLAIR	Fluid-attenuated Inversion Recovery
GCP	Good Clinical Practice
HCG	Human chorionic gonadotropin
HIPAA	Health Insurance Portability and Accountability Act of 1996

ICF	Informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IIT	Investigator-initiated trial
IEAS	Imaging Evaluable Analysis Set
IMP	Investigational medicinal product
IRB	Institutional Review Board
IV	Intravenous
LAR	Legally authorized representative
LAT1	L-type Amino Acid Transporter 1
MBq	MegaBecquerel
mCi	Millicurie
MedDRA	Medical Dictionary for Regulatory Activities
MR	Magnetic resonance
MRI	Magnetic resonance imaging
NCCN	National Comprehensive Cancer Network
NPV	Negative predictive value
PET	Positron emission tomography
PPV	Positive predictive value
RANO	Response Assessment in Neuro-Oncology
ROC	Receiver operating characteristic
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAF	Safety Analysis Set
SoC	Standard of care
SoT	Standard of truth

SRS	Stereotactic radiosurgery
SUSAR	Suspected Unexpected Serious Adverse Reaction
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
USA	United States of America

PROTOCOL SYNOPSIS

Study 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)
Phase	2b
Sponsor	Blue Earth Diagnostics, Ltd
Funding Organization	Blue Earth Diagnostics, Ltd
Study Design	This is a prospective, open-label, single-arm, single-dose study in patients 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.
Study Rationale	Brain metastases are the most common intracranial tumor in adults, occurring in up to 40% of patients with cancer (Suh, 2010; Sinha, 2017), with approximately 200,000 patients affected each year in the USA (Arvold, 2016). Following localized treatment of brain metastases (increasingly, stereotactic radiosurgery [SRS] alongside neurosurgical resection [Kann, 2017; NCCN, 2019]), close follow-up with serial magnetic resonance imaging (MRI) of the brain is performed to evaluate for recurrent disease. Conventional MRI is currently recommended as the main imaging test (NCCN, 2019) following localized treatment, as it is widely available and offers high spatial resolution, with presence of recurrent disease suggested by increased contrast enhancement (CE) depicting anatomical/structural information. However, conventional MRI (CE-T_1 and fluid attenuated inversion recovery [FLAIR]/T_2-weighted sequences) has limited specificity due to the incidence of treatment-related changes, including radiation necrosis (Galldiks, 2019). These treatment-related changes have similar appearance to true recurrence of disease on conventional MRI, including CE, origin near the primary tumor site, vasogenic edema, growth over time, and mass effect (Langen, 2017; Langen, 2018; Pope, 2018). No specific feature or combination of features on conventional MRI has been established to differentiate between disease recurrence and treatment-related changes including radiation necrosis and pseudoprogression (Verma, 2013). Specificity of conventional MRI to diagnose recurrent tumor after SRS using visual reads has been reported to be as low as 19% (Peng, 2018), with attempts at validating permutations of neuroradiologist-defined measurements reporting specificities of 32% to 41% (Dequesada, 2008; Stockham, 2012). Alongside the estimated 25% incidence rate of radiation necrosis (Vellayappan, 2018), rates of true local recurrence of disease are similar, ranging from 27% to 31% (Kocher, 2011; Brown, 2010). Therefore, the true prevalence of recurrent disease post-radiotherapy where

	conventional MRI indicates the possibility of recurrence, can be estimated to be approximately 50%. Given this area of great diagnostic unmet need, accurate imaging to differentiate disease recurrence from treatment-related changes is valuable for several reasons: • Identifying treatment-related changes is important to avoid unneeded treatment (e.g., surgery) and erroneously premature termination of potentially effective treatment (Walker, 2014). • Accurate scans can inform the management decision of cessation of non-effective treatments, to minimize morbidity from treatment side effects (Galldiks, 2019) and reduce the economic burden. • Timely diagnosis of true recurrence will allow prompt stratification of patients to further therapies (Galldiks, 2019), which may maximize therapeutic benefit and clinical outcome. • Given the high morbidity and mortality of patients with brain metastases and, therefore, the need for high quality clinical research, such imaging will be pivotal in determining suitability for clinical trial entry, and accurate characterization of investigational therapeutic efficacy (Lin, 2015). This particular need for certainty on optimal clinical trial endpoints has been recognized by the Food and Drug Administration (FDA) and National Brain Tumor Society (NBTS), leading to recent joint efforts to stimulate much needed research and development in this area (FDA/NBTS, 2019). • Aiding the physician to risk-stratify continuation of a therapeutic regimen (where treatment-related changes can be confidently diagnosed). This is of particular value in the context of a treatment with a significant side effect profile. Thus, the investigation of ^{18}F-fluciclovine in the imaging of brain metastases is of considerable clinical relevance. The ability of ^{18}F-fluciclovine to detect prostate cancer recurrence when used as a PET imaging agent has been confirmed and it is approved for clinical use in this condition. ^{18}F-fluciclovine is also reported to have utility in the evaluation of primary and metastatic cancers in the brain due to its low normal brain background uptake and increased uptake by brain tumors (Parent, 2018; Patel, 2018; Tsuyuguchi, 2017). In particular, one of these studies was performed specifically in patients with previously treated brain metastases and an MRI indeterminate for recurrence. The study reported increased ^{18}F-fluciclovine uptake in all lesions with

	recurrence, and low ^{18}F-fluciclovine uptake in lesions with treatment-related change (Patel, 2018). ^{18}F-fluciclovine has been demonstrated to be transported by amino acid transporters alanine-serine-cysteine-transporter 2 (ASCT2) and L-type amino acid transporter 1 (LAT1), which are high-affinity glutamine and leucine transporters, respectively (Schuster, 2014). Given the known increased expression of ASCT2 and LAT1 in many tumor types (including the 3 most common sources of brain metastases: lung, breast and skin cancer) (Fuchs, 2005), and aforementioned early clinical study data, it is anticipated that PET imaging with ^{18}F-fluciclovine will be useful in detecting recurrent brain metastases.	
Primary Objectives and Outcome Measures	**Objective:** To establish visual image interpretation criteria for ^{18}F-fluciclovine PET in detecting recurrent brain metastases.	**Outcome Measure:** 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 standard of truth (SoT) derived from central histopathological analysis of all lesions, at subject-level and lesion-level.
Secondary Objectives and Outcome Measures	**Objectives:** To establish other image interpretation criteria for ^{18}F-fluciclovine PET in detecting recurrent brain metastases.	**Outcome Measures:** 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.
	To assess subject-level diagnostic performance of ^{18}F-fluciclovine PET in detecting recurrent brain metastases.	Subject-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.

	To assess lesion-level diagnostic performance of ^{18}F-fluciclovine PET in detecting recurrent brain metastases.	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.
	To assess the safety of ^{18}F-fluciclovine injection in the patient population.	Safety of ^{18}F-fluciclovine injection in the patient population.
Study Sites	Approximately 10 sites / US Only	
Investigational Medicinal Product(s), including Control Products	**Investigational Medicinal Product**: ^{18}F-fluciclovine injection, 185 MBq (5 mCi) ± 20%, delivered as an intravenous bolus **Control Product**: Not applicable.	
Study and Subject Duration	**Study Duration**: Up to 9 months **Subject Duration***:* 2 months per subject (1 day for adverse event [AE] monitoring following ^{18}F-fluciclovine PET scan)	
Subject Population	Subjects with solid tumor brain metastases previously treated with radiation therapy who meet the inclusion and exclusion criteria will be eligible for participation in this study.	
Subject Selection	**Inclusion Criteria:** 1. Male or female ≥18 years of age at Screening (Visit 1). 2. Eastern Cooperative Oncology Group (ECOG) performance status 0-2. 3. Subject or subject's Legally Authorized Representative (LAR) is willing and able to provide written informed consent. 4. Previous history of solid tumor brain metastasis of any origin. 5. Histopathological confirmation of the primary solid tumor or a metastatic site within 4 years of Screening (Visit 1). Cancer of unknown primary is excluded. 6. Previous radiation therapy of brain metastatic lesion(s) completed at least 8 weeks before Screening. Form of radiotherapy may include stereotactic radiosurgery or whole brain radiotherapy, previously delivered as primary (initial) treatment, in the adjuvant setting (e.g., post-surgery), or in the salvage setting (repeat radiation therapy), with or without previous or concurrent systemic treatments. 7. A reference lesion considered by the site investigator to be equivocal for recurrent brain metastasis, as determined by	

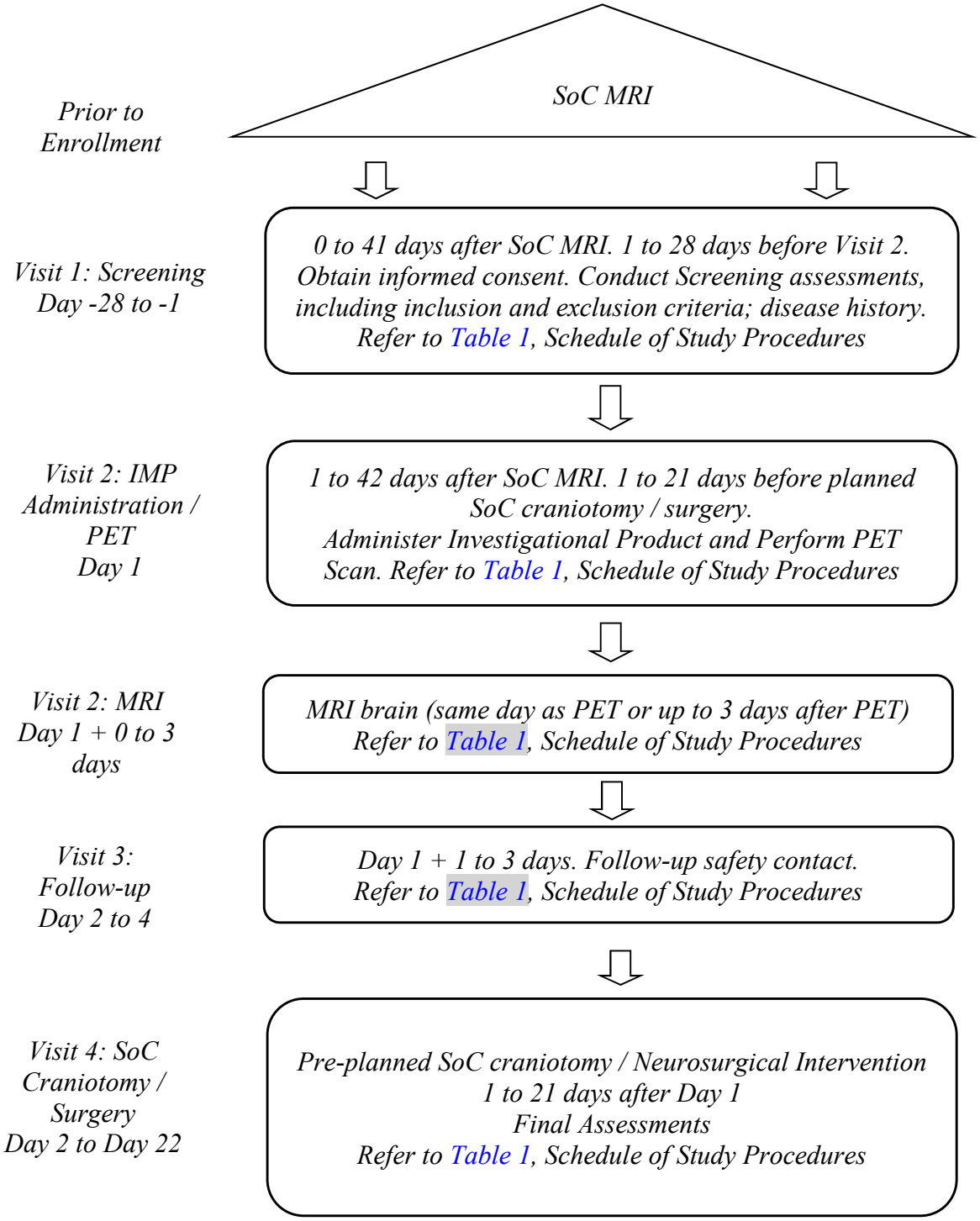
	a recent* standard of care (SoC) MRI brain scan**, measuring ≥5mm in longest diameter on contrast-enhanced MRI, and meeting one of the following radiological criteria: a. ≥20% increase in the longest diameter of the reference lesion on contrast-enhanced MRI relative to nadir***, where the longest diameter at nadir is ≥10mm b. ≥3mm increase in the longest diameter of the reference lesion on contrast-enhanced MRI relative to nadir, where the longest diameter at nadir is <10mm c. a contrast enhancing lesion at site of previous radiotherapy following complete lesion response (CLR, defined as disappearance of the irradiated contrast enhancing lesion on contrast-enhanced MRI). **SoC MRI brain scan must be completed within no more than 42 days before study PET scan.* ***The SoC MRI brain scan must consist of conventional sequences, defined as:* – *T_1-weighted without and with contrast enhancement, and* – *FLAIR and/or T_2-weighted.* *If SoC also includes advanced/investigational MRI sequences, including but not limited to perfusion, spectroscopy, diffusion weighted imaging, and susceptibility weighted imaging, trial entry is permitted as long as the other inclusion criteria are met.* ****Nadir is defined as the smallest size after radiotherapy, measured by single longest diameter on an available contrast-enhanced MRI.* 8. Subject requires further confirmatory diagnostic procedures to confirm brain MRI findings and is planned for craniotomy as SoC. 9. Females of child-bearing potential (CBP) to have negative pregnancy test (urine) before on-study ^{18}F-fluciclovine PET scan. Note: Women of child-bearing potential are defined as sexually mature women without prior hysterectomy or who have had any evidence of menses in the past 12 months. However, women who have been amenorrheic for 12 or more months are still considered to be of child-bearing potential if the amenorrhea is possibly due to prior chemotherapy, anti-estrogens, or ovarian suppression.

	10. All subjects, male and female, who are not surgically sterilized or postmenopausal as defined above, must agree to abstain from sexual conduct for 24 hours post-^{18}F-fluciclovine injection. **Exclusion Criteria:** 1. Pregnant or breastfeeding during participation in the study, or, where applicable, unwilling to abstain from sexual conduct for 24 hours post-^{18}F-fluciclovine injection. 2. Subjects with any medical condition or circumstance that the investigator believes may confound the data collected. 3. Subjects with a history of active hematological malignancy or cancer of unknown primary. 4. Subject has received, or is scheduled to receive, another investigational medicinal product (IMP) from 1 month or within 5 half-lives of the other IMP (whichever is shorter) before ^{18}F-fluciclovine injection to the completion of Visit 3.* **Subjects receiving concurrent systemic therapies, including immunotherapies, are permitted for study entry as long as the concurrent therapy is not an IMP.* 5. Known contraindications to a contrast-enhanced MRI procedure. 6. Applicable to subjects actively on the BED-FLC-312 study: the subject at time of Screening, is within the BED-FLC-312 Visit 4 window (i.e. up to and including Day 22).
Planned Interim Analyses	Not applicable.
STATISTICS **Primary Analysis Plan**	Diagnostic performance (sensitivity, specificity, PPV and 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, will be calculated. 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. Receiver operating characteristic (ROC) analysis of SoT against the quantitative measures of lesion ^{18}F-fluciclovine uptake will be performed. For dynamic measures of lesion ^{18}F-fluciclovine uptake represented by time activity curve (TAC) type, diagnostic performance for each TAC category will be calculated. The Image Interpretation Committee will review these analyses to establish appropriate image interpretation criteria. Subsequently, for the diagnostic performance secondary endpoints, sensitivity, specificity, PPV and NPV for a

	combination of the established image interpretation criteria will be calculated based on the recommended thresholds, using standard definitions. 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). For the evaluation of safety, 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, through 1 day following IMP administration. Adverse event verbatim terms will be mapped to preferred terms and system organ classes using the current Medical Dictionary for Regulatory Activities (MedDRA). Severity of AEs will be graded using the NCI- Common Terminology Criteria for Adverse Events (CTCAE) v5.0. The frequency of TEAEs will be summarized overall, by system organ class and preferred term, and by severity grade and relationship to IMP.
Rationale for Number of Subjects	Up to 40 subjects will be enrolled. Sufficient numbers of subjects will be included in the study in order to gain sufficient data to establish image interpretation criteria. No formal statistical hypotheses are stated.

STUDY SCHEMA

Figure 1. Schema of Study Design



IMP, investigational medicinal product; MRI, magnetic resonance imaging; PET, positron emission tomography; SoC, standard of care.

1. BACKGROUND

^{18}F-fluciclovine injection is an investigational medicinal product (IMP) for positron emission tomography (PET) imaging with the active ingredient ^{18}F-labeled 1-amino-3-fluorocyclobutane-1-carboxylic acid in the '*anti*' configuration. The unlabeled substance, as manufactured by the inventors, Professor Goodman's group at Emory University, Atlanta, Georgia, United States of America (USA), is commonly referred to as FACBC and is commercially known as Axumin® (fluciclovine F 18). ^{18}F-fluciclovine injection is prepared as a ready-to-inject solution. [redacted]

^{18}F-fluciclovine is a synthetic amino acid that is actively transported into mammalian cells by amino acid transporters, but is not then incorporated into newly synthesized proteins. Following injection, ^{18}F-fluciclovine is preferentially taken up into cells/tissues with enhanced amino acid transport, such as tumor cells that require increased amounts of amino acids to support increased metabolism and proliferation. Positron emission tomography imaging studies have demonstrated that ^{18}F-fluciclovine is preferentially taken up into prostate carcinoma and primary brain tumors compared with surrounding normal tissue (Bach-Gansmo, 2017; Bogsrud, 2019; Michaud, 2020). In addition, in vitro studies have shown that uptake of ^{14}C-fluciclovine in C6 glioma cells is higher than that seen in granulocytes/macrophages. This may be advantageous in discriminating inflamed regions from tumors (Oka, 2014).

^{18}F-fluciclovine is licensed in the USA and European Union (EU) for PET imaging in biochemical recurrence of prostate cancer (NDA 208,054, approved 27 May 2016; EMEA/H/C/004197; approved 22 May 2017). ^{18}F-fluciclovine has a well-established safety profile and a wide distribution network in the USA where it is routinely used as standard of care (SoC) for imaging in biochemical recurrence of prostate cancer.

Blue Earth Diagnostics, Ltd (BED) is developing ^{18}F-fluciclovine injection as a diagnostic radiopharmaceutical for PET imaging to visualize increased amino acid transport that occurs in malignant tumors, including in brain metastases with suspected recurrence after radiation therapy.

^{18}F-fluciclovine has been demonstrated to be transported by amino acid transporters alanine-serine-cysteine transporter 2 (ASCT2) and L-type amino acid transporter 1 (LAT1), which are high-affinity glutamine and leucine transporters, respectively (Oka, 2012; Okudaira, 2011; Ono, 2013; Teoh, 2017).

^{18}F-fluciclovine is reported to have characteristics favorable for use as a PET imaging agent in the evaluation of primary and metastatic cancers in the brain, including low brain background uptake, and high uptake described in primary and secondary malignant brain tumors (Schuster, 2003; Karlberg, 2019; Michaud, 2020; Parent, 2018; Patel, 2018; Tsuyuguchi, 2017).

Given these favorable imaging characteristics, and the known expression of amino acid transporters in many tumor types (including the 3 most common sources of brain metastases: lung, breast and skin cancer) (Fuchs, 2005), it is anticipated that amino acid PET imaging with ^{18}F-fluciclovine will be useful in detecting brain metastases where recurrence is suspected following radiation therapy.

1.1. Overview of Clinical Studies

Two small investigator-initiated trials (IITs) conducted at Emory University have evaluated ^{18}F-fluciclovine for PET imaging of brain metastases in adults (Schuster, 2003; Patel, 2018), with results suggesting efficacy. Both studies reported increased ^{18}F-fluciclovine uptake in brain metastases: one study in 5 patients with 9 lesions and another in 12 patients with 17 lesions. In the larger study, all patients had prior treatment for brain metastases and had a magnetic resonance imaging (MRI) indeterminate for recurrent disease. Using either biopsy or clinical follow-up as the standard of truth (SoT), 15/15 ^{18}F-fluciclovine positive lesions were determined to be recurrent disease. On the other hand, the remaining 2 lesions were determined to be radiation necrosis, both of which had low ^{18}F-fluciclovine uptake.

Apart from brain metastases, other studies have also reported the use of ^{18}F-fluciclovine in other neuro-oncology settings (Parent, 2018; Karlberg, 2019; Michaud, 2020; Bogsrud, 2019). This includes one IIT in suspected recurrent primary brain tumor (N=27) (Michaud, 2020), where tumor uptake of ^{18}F-fluciclovine provided significantly higher image contrast to ^{11}C-Methionine. ^{18}F-fluciclovine, based on a subset of 3 patients with indeterminate MRI, was suggested to be potentially useful in this setting. A retrospective analysis of 21 patients with suspected residual or recurrent high grade glioma (Bogsrud, 2019) reported high ^{18}F-fluciclovine uptake in histopathologically / clinically confirmed disease, including the detection of small satellite tumors not previously visualized with MRI in 4 patients. A recent report of 2 cases has also described the successful use of ^{18}F-fluciclovine PET-MRI–guided biopsy in post-treatment glioblastoma to distinguish areas of tumor recurrence from areas of predominant treatment effect (Henderson, 2019). Industry sponsored studies include 4 studies conducted by Nihon Medi-Physics Co., Ltd (NMP) in patients with treatment-naïve glioma: NMK36-BT-P201 (N=5) (Kondo, 2016), MK36-BT-P202 (N=40) (Wakabayashi, 2017), NMK36-BT-P301 and NMK36-BT-P302. These studies collectively suggest the potential for ^{18}F-fluciclovine to delineate extent of treatment-naïve primary brain tumor beyond contrast-enhancing regions on MRI.

As a marketed product, ^{18}F-fluciclovine has been administered to more than 75,000 patients. Safety data are available from 1203 patients exposed to ^{18}F-fluciclovine in clinical trials. The doses administered range from 51.8 to 485 MBq, with the majority of patients receiving approximately 370 MBq for the imaging of prostate cancer, and approximately 180 MBq for glioma in Studies NMK36-BT-P201, NMK36-BT-P202, NMK36-BT-P301 and NMK36-BT-P302. No deaths and no serious adverse events (SAEs) attributable to ^{18}F-fluciclovine injection have been reported. Adverse events (AEs) have generally been mild in intensity. Based on the accumulated information, injection site reactions, dysgeusia and parosmia are considered to be adverse reactions associated with receipt of ^{18}F-fluciclovine injection in humans; these events occurred in 17/1203 (1.4%) patients overall.

Based upon preliminary data from the 2 IITs conducted at Emory University (Schuster, 2003; Patel, 2018), and supported by experience reported from the collective clinical studies of ^{18}F-fluciclovine in other brain tumor settings (Parent, 2018; Karlberg, 2019; Michaud, 2020; Bogsrud, 2019; Henderson, 2019), BED is conducting the current clinical study (BED-FLC-219) as the Phase 2 study in a clinical program to support the use of ^{18}F-fluciclovine PET imaging to detect brain metastases in adults with suspected recurrence after radiation therapy.

2. STUDY RATIONALE

Brain metastases are the most common intracranial tumor in adults, occurring in up to 40% of patients with cancer (Suh, 2010; Sinha, 2017), with approximately 200,000 patients affected each year in the US (Arvold, 2016). Following localized treatment of brain metastases (increasingly, stereotactic radiosurgery [SRS] alongside neurosurgical resection [Kann, 2017; NCCN, 2019]), close follow-up with serial MRI of the brain is performed to evaluate for residual and recurrent disease. Conventional MRI is currently recommended as the main imaging test (NCCN, 2019) following localized treatment, as it is widely available and offers high spatial resolution, with presence of recurrent disease suggested by increase contrast enhancement (CE) depicting anatomical/structural information. However, conventional MRI (CE-T_1 and fluid-attenuated inversion recovery [FLAIR]/T_2-weighted sequences) has limited specificity due to the incidence of treatment-related changes, primarily radiation necrosis (Galldiks, 2019). These treatment-related changes have similar appearances to true recurrence of disease on conventional MRI, including CE, origin near the primary tumor site, vasogenic edema, growth over time, and mass effect (Langen, 2017; Langen, 2018; Pope, 2018).

No specific feature or combination of features on conventional MRI has been established to differentiate between disease recurrence and treatment-related changes, including radiation necrosis and pseudoprogression (Verma, 2013). Specificity of conventional MRI to diagnose recurrent tumor after SRS using visual reads has been reported to be as low as 19% (Peng, 2018), with attempts at validating permutations of neuroradiologist-defined measurements reporting specificities of 32% to 41% (Dequesada, 2008; Stockham, 2012). Alongside the estimated 25% incidence rate of radiation necrosis (Vellayappan, 2018), rates of true local recurrence of disease are similar, ranging from 27% to 31% (Kocher, 2011; Brown, 2010). Therefore, the true prevalence of recurrent disease post-radiotherapy where conventional MRI indicates the possibility of recurrence, can be estimated to be approximately 50%.

Guidelines and recommendations established by the Response Assessment in Neuro-Oncology (RANO) group recognize the limitations of conventional MRI, and in view of the lack of substantial evidence, do not yet advocate any advanced imaging technique when MRI features are suggestive, but equivocal, for recurrence of brain metastases (Wen, 2010; Lin, 2015). The management of patients with MRI findings suggestive, but equivocal, for recurrent brain metastases includes clinical monitoring or biopsy sampling (Lin, 2015). Close clinical monitoring with repeat imaging may be performed in the hope that scan appearance resolves over time, but this is at the expense of delayed therapeutic decision making. Further, neurosurgical biopsy sampling is an invasive procedure that carries the associated risk of further deteriorating quality-of-life for patients already undergoing aggressive treatment (of their primary cancer) (Verma, 2013).

National Comprehensive Cancer Network (NCCN) guidelines list advanced MRI scans (magnetic resonance [MR] spectroscopy, MR perfusion) and PET imaging as potentially useful techniques in differentiating tumor from treatment effect (NCCN, 2019); however, the availability, acquisition, processing, interpretation and, therefore, diagnostic performance of advanced MRI scans vary considerably (Zhang, 2017). In addition, the only PET imaging agent approved by the US Food and Drug Administration (FDA) for use in brain tumor imaging is ^{18}F-fluorodeoxyglucose (FDG), which has notable limitations. Use of ^{18}F-FDG in brain tumor imaging is limited in sensitivity and specificity, as differentiation of tumor from non-tumorous tissue is often difficult due to the high metabolic rate of normal brain parenchyma and inflammatory tissue (Galldiks, 2019). ^{18}F-FDG uptake in low-grade tumors can be similar to that in normal grey matter. The sensitivity of detection of lesions is further

decreased by the high variance of ^{18}F-FDG uptake and its heterogeneity within a single tumor (Heiss, 2014).

Given this area of great diagnostic unmet need, accurate imaging to differentiate disease recurrence from treatment-related changes is valuable for several reasons:

- Identifying treatment-related changes is important to avoid unneeded treatment (e.g., surgery) and erroneously premature termination of potentially effective treatment (Walker, 2014).
- Accurate scans can inform the management decision of cessation of non-effective treatments, to minimize morbidity from treatment side effects (Galldiks, 2019) and reduce the economic burden.
- Timely diagnosis of true recurrence will allow prompt stratification of patients to further therapies (Galldiks, 2019), which may maximize therapeutic benefit and clinical outcome.
- Given the high morbidity and mortality of patients with brain metastases and, therefore, the need for high quality clinical research, such imaging will be pivotal in determining suitability for clinical trial entry, and accurate characterization of investigational therapeutic efficacy (Lin, 2015). This particular need for certainty on optimal clinical trial endpoints has been recognized by the FDA and National Brain Tumor Society (NBTS), leading to recent joint efforts to stimulate much needed research and development in this area (FDA/NBTS, 2019).
- Aiding the physician to risk-stratify continuation of a therapeutic regimen (where treatment-related changes can be confidently diagnosed). This is of particular value in the context of a treatment with a significant side effect profile.

Limited clinical research has been conducted with other amino acid PET tracers in brain metastases, possibly in part due to highly limited availability in the US. Studies of brain metastases with amino acid PET tracers ^{18}F-fluoro-ethyltyrosine (^{18}F-FET), ^{18}F-fluoro-dihydroxyphenylalanine (^{18}F-FDOPA) and ^{11}C-methionine have all been described, with demonstration of increased tracer uptake and sensitivities of 79% to 95%, specificities of 84% to 93% for the detection of brain metastases where MRI is suggestive of recurrent disease (Terakawa, 2008; Galldiks, 2012;Lizarraga, 2014; Cicone, 2015; 2015; Romagna, 2016; Ceccon, 2017). In recent recommendations by the RANO working group, amino acid PET was recommended as useful in distinguishing post-therapeutic reactive changes following radiotherapy from recurrent brain metastases (Galldiks, 2019). This was based on review of Level 2 evidence on ^{18}F-FET, ^{18}F-FDOPA and ^{11}C-methionine. Transport mechanisms of these tracers have overlap with ^{18}F-fluciclovine, in that these are predominantly LAT1 mediated. Furthermore, known cross-affinity for various amino acids between the 2 transporters support the expectation for ^{18}F-fluciclovine to demonstrate comparable performance in brain metastases, akin to other amino acid PET tracers and known ^{18}F-fluciclovine imaging characteristics in glioma. Thus, the investigation of ^{18}F-fluciclovine in the imaging of brain metastases is of considerable clinical relevance, particularly when considering promising results from the aforementioned IITs of ^{18}F-fluciclovine in brain metastases,

Amongst the previously cited studies using ^{18}F-FET, ^{18}F-FDOPA and ^{11}C-methionine, different interpretation criteria for optimum detection of disease recurrence have been reported. Furthermore, these criteria have been based on different methods of quantifying PET tracer uptake, and in the case of ^{18}F-FET, incorporate the interpretation of dynamic scan

data (time-activity curves). Therefore, existing heterogeneous data on optimum image interpretation criteria in other amino acid PET tracers cannot be directly applied to ^{18}F-fluciclovine.

The purpose of the current study is to take initial steps to address this area of diagnostic unmet need by defining image interpretation criteria for ^{18}F-fluciclovine PET in detecting recurrent brain metastases in adults with suspected recurrence after radiation therapy.

2.1. Risk-Benefit Assessment

2.1.1. Benefit

The ^{18}F-fluciclovine PET scans may provide further clinical information regarding the patient's disease status that may not have been appreciated using other standard of care tests. If such information arises, this may be considered by the responsible clinician to help direct the patient's management, including guiding the planned surgery. This may provide a direct benefit to the subject.

2.1.2. Risk

The risks from the imaging studies to patients mainly relate to the intravenous (IV) injection and the radiation emitted by the radiopharmaceutical and the CT transmission scan (when the PET scan is acquired on a PET/CT scanner). There is a potential risk of a hypersensitivity reaction requiring the availability of resuscitative equipment and personnel. Intravenous injection carries a small risk of infection and hematoma.

The mean effective dose per unit administered activity of ^{18}F-fluciclovine is 22.1 µSv/MBq (McParland, 2013). An administered activity of 5 mCi (185 MBq) will result in an effective dose of 4.1 mSv. If PET/CT is used, the effective dose due to CT acquisition should be in accordance with ALARA (as low as reasonably achievable) principles. The maximum effective dose due to a CT transmission scan will vary from site to site, but as a guide, a dose ranging from 0.5 mSv to 2.0 mSv would be expected. Combined with the effective dose from ^{18}F-fluciclovine (4.1 mSv), the total effective dose of the ^{18}F-fluciclovine PET-CT scan of 6.1 mSv is in line with other common brain nuclear medicine procedures.

Due to the risk of radiation exposure, women who are either pregnant or breast feeding are excluded from participation. All subjects, male and female, who are not surgically sterilized or postmenopausal, must agree to abstain from sexual conduct for 24 hours post-^{18}F-fluciclovine injection.

Image interpretation errors can occur with ^{18}F-fluciclovine PET brain imaging. Physiological distribution of ^{18}F-fluciclovine does not rule out the presence of recurrent metastasis and ^{18}F-fluciclovine uptake within a lesion does not confirm the presence of recurrent metastasis. ^{18}F-fluciclovine uptake is not specific for brain metastases and may occur with other types of malignancy in or around the brain. There may be uptake in other conditions such as active multiple sclerosis, brain abscess, and meningioma. Accordingly, as the responsible clinician may consider information from the ^{18}F-fluciclovine PET scan to direct the patient's management, there is a potential risk of additional resections/biopsies including those that are ultimately unnecessary.

3. STUDY OBJECTIVES

3.1. Primary Objective

The primary objective is

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

3.2. Secondary Objectives

The secondary objectives 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 patient population.

4. STUDY DESIGN

4.1. Study Overview

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 MRI for anatomical reference).

Subjects with a history of brain metastases previously treated with primary, adjuvant or repeat (salvage) radiation therapy, with a recent 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, see 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 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 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 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.

4.2. 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.

5. CRITERIA FOR EVALUATION

5.1. Primary Efficacy Endpoint

- 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.

5.2. Secondary Efficacy Endpoints

- 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.
- Safety of ^{18}F-fluciclovine injection in the patient population.

5.3. Safety Evaluations

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).

6. SUBJECT SELECTION

6.1. Study Population

Subjects with solid tumor brain metastases previously treated with radiation therapy who are scheduled for SoC craniotomy and meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2. Inclusion Criteria

1. Male or female ≥18 years of age at Screening (Visit 1).
2. Eastern Cooperative Oncology Group (ECOG) performance status 0-2.
3. Subject or subject's Legally Authorized Representative (LAR) is willing and able to provide written informed consent.
4. Previous history of solid tumor brain metastasis of any origin.
5. Histopathological confirmation of the primary solid tumor or a metastatic site within 4 years of Screening (Visit 1). Cancer of unknown primary is excluded.
6. Previous radiation therapy of brain metastatic lesion(s) completed at least 8 weeks before Screening. Form of radiotherapy may include stereotactic radiosurgery or whole brain radiotherapy, previously delivered as primary (initial) treatment, in the adjuvant setting (e.g., post-surgery), or in the salvage setting (repeat radiation therapy), with or without previous or concurrent systemic treatments.
7. A reference lesion considered by the site investigator to be equivocal for recurrent brain metastasis, as determined by a recent* standard of care (SoC) MRI brain scan**, measuring ≥5mm in longest diameter on contrast-enhanced MRI, and meeting one of the following radiological criteria:
   a. ≥20% increase in the longest diameter of the reference lesion on contrast-enhanced MRI relative to nadir***, where the longest diameter at nadir is ≥10mm
   b. ≥3mm increase in the longest diameter of the reference lesion on contrast-enhanced MRI relative to nadir, where the longest diameter at nadir is <10mm
   c. a contrast enhancing lesion at site of previous radiotherapy following complete lesion response (CLR, defined as disappearance of the irradiated contrast enhancing lesion on contrast-enhanced MRI).

   **SoC MRI brain scan must be completed within no more than 42 days before study PET scan.*

   ***The SoC MRI brain scan must consist of conventional sequences, defined as:*

   – *T_1-weighted without and with contrast enhancement, and*

   – *FLAIR and/or T_2-weighted.*

   If SoC also includes advanced/investigational MRI sequences, including but not limited to perfusion, spectroscopy, diffusion weighted imaging, and susceptibility weighted imaging, trial entry is permitted as long as the other inclusion criteria are met.

[***]Nadir is defined as the smallest size after radiotherapy, measured by single longest diameter on an available contrast-enhanced MRI.

8. Subject requires further confirmatory diagnostic procedures to confirm brain MRI findings and is planned for craniotomy as SoC.
9. Females of child-bearing potential (CBP) to have negative pregnancy test (urine) before on-study ^{18}F-fluciclovine PET scan.

   Note: Women of child-bearing potential are defined as sexually mature women without prior hysterectomy or who have had any evidence of menses in the past 12 months. However, women who have been amenorrheic for 12 or more months are still considered to be of child-bearing potential if the amenorrhea is possibly due to prior chemotherapy, anti-estrogens, or ovarian suppression.
10. All subjects, male and female, who are not surgically sterilized or postmenopausal as defined above, must agree to abstain from sexual conduct for 24 hours post-^{18}F-fluciclovine injection.

6.3. Exclusion Criteria

1. Pregnant or breastfeeding during participation in the study, or, where applicable, unwilling to abstain from sexual conduct for 24 hours post-^{18}F-fluciclovine injection.
2. Subjects with any medical condition or circumstance that the investigator believes may confound the data collected.
3. Subjects with active hematological malignancy or cancer of unknown primary.
4. Subject has received, or is scheduled to receive, another IMP from 1 month or within 5 half-lives of the other IMP (whichever is shorter) before ^{18}F-fluciclovine injection to the completion of Visit 3.[*]

   [*]Subjects receiving concurrent systemic therapies, including immunotherapies, are permitted for study entry as long as the concurrent therapy is not an IMP.
5. Known contraindications to a contrast-enhanced MRI procedure.
6. Applicable to subjects actively on the BED-FLC-312 study: the subject at time of Screening, is within the BED-FLC-312 Visit 4 window (i.e., up to and including Day 22).

7. CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

7.1. Allowed Medications and Treatments

Standard therapy for solid tumor brain metastases (e.g., systemic therapies, including immunotherapies) is allowed except for treatments noted in the exclusion criteria described above and as noted in the prohibited medications section below.

7.2. Prohibited Medications and Treatments

The following medications are prohibited during the study and administration will be considered a protocol violation:

- any concurrent therapy that is an IMP, prohibited from 1 month or within 5 half-lives of the other IMP (whichever is shorter) before ^{18}F-fluciclovine injection to the completion of Visit 3.

8. STUDY TREATMENTS

8.1. Method of Assigning Subjects to Treatment Groups

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

8.2. Blinding

Not applicable.

8.3. Formulation of Test Product

^{18}F-fluciclovine injection is a fluorine-18 labelled synthetic amino acid PET diagnostic agent supplied as a ready-to-inject solution. [redacted]

^{18}F-fluciclovine injection is manufactured by automated radiosynthesis followed by formulation with buffer and aseptic dispensing in a remotely controlled system.

The radioactive isotope Fluorine-18 (^{18}F) decays by positron emission (β+ decay, 96.7%) and orbital electron capture (3.3%) with a half-life of approximately 110 minutes.

8.4. Packaging and Labelling

The investigational agent is supplied as a unit dose for injection in a syringe with a radioactive concentration at a reference date and time that is stated on the container label. Each syringe is supplied in a container providing appropriate radiation shielding. Information will be provided with the shipment giving the confirmation number, radioactive concentration of injection (mCi/mL) at a stated time and date, shelf life information, protocol number and a unique prescription number. The radiochemical purity of ^{18}F-fluciclovine injection is not less than 95% during the shelf life of the product.

8.5. Supply of Study Drug at the Site

Sites will be provided with instructions for ordering the ^{18}F-fluciclovine doses for use in the study. The specific date and time for a subject to be scanned needs to be included when placing the order. Site should be aware that next-day delivery of the ^{18}F-fluciclovine is not feasible in all circumstances. If a site intends to administer the ^{18}F-fluciclovine dose the day after ordering, they should first confirm the order and delivery time with the study IMP administrators. The ^{18}F-fluciclovine will be delivered from the radiopharmacy to the imaging site by courier. Each syringe is supplied in a container providing appropriate radiation shielding. The site must keep records of all shipments of ^{18}F-fluciclovine received, dispensing and disposal/destruction performed on site as is appropriate to each facility.

Additional details concerning ordering and handling of the IMP are provided in the Pharmacy Manual and Image Acquisition Standards.

8.6. Dosage/Dosage Regimen

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

8.7. Dispensing

When the study site receives the dose, both prior to and after administration, the activity in the syringe will be measured using an appropriate dose calibrator. Should the activity be less than 148 MBq (4 mCi) or the volume required exceed 5 mL of undiluted material or the administration be after the labeled expiration time, the scan should not be performed.

Additional details for handling the IMP are provided in the Pharmacy Manual.

8.8. Administration Instructions

Position the subject supine, with arms alongside the body and the head stabilized appropriately. The entire brain, including the cerebellum, should be in the field of view. A venous cannula will be inserted, and the subject will receive an administered activity of 185 MBq (5 mCi) ± 20% of ^{18}F-fluciclovine. The ^{18}F-fluciclovine will be administered as an IV bolus injection followed by a 10 mL flush of normal saline solution.

Additional details are provided in the Image Acquisition Standards.

8.9. Storage

The shelf-life of ^{18}F-fluciclovine injection is up to 10 hours from the end of production and the product must not be administered beyond this limit. ^{18}F-fluciclovine should be stored at 15 to 25°C in a shielded container.

All non-radioactive containers (shielding, transport cans) must be returned to the manufacturing site. Shipping containers that are radioactive must be decontaminated or allowed to decay prior to return to the manufacturing site. Used product syringes and other radioactive materials must be destroyed at either the study site or another designated facility.

Waste must be disposed of according to national and local regulations for radioactive material.

Precautions for the safe handling of radioactive materials should be observed.

Additional details are provided in the Pharmacy Manual.

8.10. Study Drug Accountability

An accurate and current accounting of the dispensing and disposal of study drug for each subject will be recorded on the Investigational Drug Accountability Record. These records will be maintained at the radiopharmacy, hot lab or nuclear medicine department responsible for receipt and dispensation of study drug. Records should include at a minimum:

- Dates of receipt, lot number and quantities received from Sponsor or designee;
- Dates, subject numbers, and amount of ^{18}F-fluciclovine dispensed for administration to specific subjects, including administered dose activity;
- If applicable, for any unused IMP, dates, lot numbers, and reason dose was not administered.

The investigator is responsible for ensuring that study drug is administered only to subjects included in this study in accordance with the study protocol.

Throughout the study, drug accountability will be performed by appropriate Sponsor representative(s) and when appropriate, reconciliation will be performed. Additional details are provided in the Pharmacy Manual.

8.11. Measures of Treatment Compliance

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

8.12. End Of Trial

For each subject, the last visit will usually be Visit 4/Day 2-22 SoC craniotomy/surgery. All ongoing follow-up and any further treatment will be in accordance with SoC.

9. STUDY PROCEDURES AND GUIDELINES

A schedule of events representing the required study procedures to be performed for the duration of the study is provided in Table 1.

Prior to conducting any study-related activities, written informed consent (and assent, if applicable) including the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject or LAR (Section 17.3).

Table 1. Schedule of Study Procedures

	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		

	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
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.

9.1. Clinical Assessments

9.1.1. Concomitant Medications

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. Dose, route, frequency of administration, and indication for administration and dates of medication will be captured.

9.1.2. Demographic and Baseline Characteristics

Demographic information recorded at Screening will include age, sex, race and ethnicity. Baseline assessments of pre-scan body weight and height will be collected at Visit 2.

9.1.3. ECOG Performance Status

Conduct ECOG Performance Status assessment at Screening (Visit 1) or record a standard of care ECOG Performance Status if the assessment was performed within 28 days prior to Screening (Visit 1) (see Appendix 1).

9.1.4. Medical History

Record medical and disease history at Screening (Visit 1). Include history for previous brain metastases and brain metastases treatments, diagnosis of primary tumor and previous treatments of primary tumor, and any other previous cancers, as applicable. Brain metastases and primary tumor histories should include dates of initial diagnosis, initial tumor stage, dates and type of definitive therapy and adjuvant treatment (if given). 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.

9.1.5. Imaging Studies

Sites will be instructed to submit historic SoC imaging data required to confirm eligibility. Submitted MRI scans should confirm the reference lesion criteria, outlined in Section 6.2. Therefore, these should be the MRI scan confirming reference lesion nadir or showing complete lesion response, the current SoC MRI brain scan performed before Screening (Visit 1), and may include the pre-treatment MRI if available. Sites will annotate and measure the reference lesion and MRI surgical lesions on the current SoC MRI brain scan using the imaging core lab study imaging platform.

The reference lesion is defined as the lesion which is:

a. equivocal for recurrent metastasis on SoC MRI (according to inclusion criterion #7, see Section 6.2);

b. intended for pre-planned SoC craniotomy; and

c. if >1 equivocal lesion is intended for resection, the largest of these lesions.

If >1 equivocal lesion is pre-planned for resection as SoC, 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 Section 6.2).

Site will perform and upload a dynamic ^{18}F-fluciclovine PET brain scan on Day 1 (Visit 2), and an on-study MRI brain scan performed 0 to 3 days after the ^{18}F-fluciclovine PET scan (Visit 2).

The site investigator will 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 according to SoC practice, be neurosurgically feasible and accordingly, planned for biopsy/resection. 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.

The investigator will then annotate the additional PET lesions on the ^{18}F-fluciclovine PET scan using the imaging core lab study imaging platform. The site investigator will also confirm the sites of the reference and MRI surgical lesions on the Visit 2 scans.

For each annotated lesion, a lesion identifier will be automatically created and assigned by the imaging core lab study imaging platform. For each annotated lesion, the site will also be required to specify the anatomical location.

9.1.5.1. ^{18}F-fluciclovine PET Scan

Dynamic PET/CT or PET/MRI scan of the brain is to be performed at Visit 2 following ^{18}F-fluciclovine injection. If the PET scan is performed on a PET/CT scanner, a CT scan of the brain will also be performed for attenuation correction. Site investigator will review the ^{18}F-fluciclovine PET scan to identify potential additional PET lesions (this will be done with the Visit 2 MRI scan for anatomical reference). The PET scan will be uploaded to central imaging core lab by site investigator/site staff. The selected PET scanner must be qualified by the study management team prior to any imaging studies. Full details on the imaging protocol are provided in the Image Acquisition Standards.

9.1.5.2. On-Study MRI

Throughout this study, ^{18}F-fluciclovine PET will be read with standard MRI for anatomical reference. Therefore, all subjects will receive an on-study, study-specific brain MRI scan at Visit 2, ideally on the same day as the PET scan or ≤3 days after the PET scan. On-study MRI will consist of MRI sequences per the minimum standard consensus recommendations for a standardized brain tumor imaging protocol for clinical trials in brain metastases (BTIP-BM) (Kaufmann, 2020). These will include:

- T_1-weighted MRI without and with contrast enhancement, and
- FLAIR and/or T_2-weighted MRI, and
- Diffusion-weighted imaging.

On-study MRI brain scan will be uploaded to central imaging core lab by site investigator/site staff. In addition to reviewing the ^{18}F-fluciclovine PET scan to identify potential additional PET lesions, the site investigator will confirm the sites of the reference lesion and if applicable, MRI surgical lesions (i.e., other lesions already planned for resection per SoC).

Full details on the imaging protocol are provided in the Image Acquisition Standards. If available, scans acquired on a PET/MRI are allowed.

9.1.6. 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.

9.1.7. SoC Craniotomy / Surgery

Craniotomy procedure to take place as planned for SoC, for surgical resection of the reference lesion and MRI surgical lesions, and biopsy / surgical resection of any additional PET lesions if clinically applicable. En bloc resection is to be performed where possible. Where en bloc resection is not possible, the surgeon should try and send as much of the sample as possible to enable optimal histopathology assessment. Care should be taken to follow the Sample Handling Laboratory Manual to label all samples sent to the site pathology laboratory, and to reflect the locations sampled and lesion identifiers assigned by the imaging core lab study imaging platform.

The site pathology laboratory will perform histopathology analysis per SoC and will send a representative set of specimens with their matching lesion identifiers to the central laboratory for analysis. Refer to Sample Handling Laboratory Manual.

9.2. Clinical Laboratory Measurements

9.2.1. Pregnancy Test

A human chorionic gonadotropin (HCG) urine pregnancy test will be obtained from female subjects who are of child-bearing 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).

10. EVALUATIONS BY VISIT

10.1. Visit 1 –Screening

Screening to take place following SoC MRI brain scan and up to 28 days before Visit 2. Visit 1 – Screening may be performed at Day -1 (1 day prior to Visit 2 PET scan) only if next-day delivery of the ^{18}F-fluciclovine dose is confirmed by the site.

1. Review the study with the subject (subject's LAR) and obtain written informed consent and HIPAA authorization and assent, if appropriate.
2. Assign the subject a unique subject number.
3. Record demographics data.
4. Record medical history.
5. Record concomitant medications.
6. Record histopathological confirmation of the primary tumor or a metastatic site within 4 years of Screening.
7. Record previous cancer treatments for primary tumor (and other previous cancers, if applicable).
8. Record previous diagnoses and treatments for brain metastases.
9. Collect and review prior pre- and post-treatment MRI brain scans as needed for investigator to confirm eligibility and current SoC MRI. Upload MRI images to central imaging core lab and annotate and measure reference lesion and MRI surgical lesions.
10. Record or assess ECOG performance status.
11. Perform urine HCG pregnancy test (female subjects of child-bearing potential only).
12. Confirm inclusion / exclusion criteria.
13. Schedule ^{18}F-fluciclovine PET scan and order IMP for delivery on day of PET scan.
14. Schedule subject for Visit 2 (IMP + PET) to occur within 42 days of SoC MRI, and provide the subject with ^{18}F-fluciclovine PET scan preparation instructions (see Section 11.1).

The reference lesion is defined as the lesion which is:

a. equivocal for recurrent metastasis on SoC MRI (according to inclusion criterion #7, see Section 6.2)

b. intended for pre-planned SoC craniotomy; and

c. if >1 equivocal lesion is intended for resection, the largest of these lesions.

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

10.2. Visit 2- Day 1: IMP Administration and ^{18}F-fluciclovine PET Scan (1 to 28 days after Screening)

Subjects who have provided informed consent and who meet all eligibility criteria will be enrolled into the study. At the time the subject is deemed to be eligible, the following activities will be performed:

1. The subject will prepare for the ^{18}F-fluciclovine PET scan according to preparation instructions (see Section 11.1).
2. Record any changes in concomitant medications and medical history since Screening (Visit 1).
3. Perform urine HCG pregnancy test (female subjects of child-bearing potential only) unless performed 1 day prior to IMP administration.
4. Perform assessment of vital signs with subject resting at least 5 minutes prior to IMP administration. The vital signs should be collected between 5 to 60 minutes before PET scan.
5. IMP + PET: Administer ^{18}F-fluciclovine injection, followed by dynamic PET scan of the brain. If the PET scan is performed on a PET/CT scanner, a CT scan of the brain will also be performed for attenuation correction.
6. Monitor and record any adverse events.
7. Perform assessment of vital signs with subject resting at least 5 minutes after PET scan. The vital signs should be collected between 5 to 60 minutes after PET scan.
8. Upload ^{18}F-fluciclovine PET brain scan to central imaging core lab.
9. Review of ^{18}F-fluciclovine PET brain scan by site investigator to identify potential additional PET lesions will be performed with the Visit 2 MRI scan (for anatomical reference), see Section 10.3.

10.3. Visit 2 – Day 1 or up to 3 days after ^{18}F-fluciclovine Administration: On-Study MRI Brain Scan

Visit 2 on-study MRI brain scan should take place on the same day as the ^{18}F-fluciclovine PET scan (Day 1) or up to 3 days after. It should take place before the Visit 4 – SoC craniotomy.

1. Perform Visit 2 on-study MRI brain scan as outlined in Section 11.5.
2. Upload Visit 2 on-study MRI brain scan to central imaging core lab
3. Review of ^{18}F-fluciclovine PET brain scan (with Visit 2 MRI for anatomical reference) by site investigator to identify potential additional PET lesions, not previously reported on SoC MRI.
4. Confirm sites of reference lesion and MRI surgical lesions on Visit 2 scans.

The on-site investigator will review the ^{18}F-fluciclovine PET scan to identify and annotate potential additional lesions, not previously reported on SoC MRI (termed ‘additional PET lesions’). Additional PET lesions should be annotated only where judged suggestive of metastasis, warranting confirmation according to SoC practice, be neurosurgically feasible and accordingly, planned for biopsy/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.

10.4. Visit 3 – Safety Follow-up (+1 to 3 days after IMP Injection/PET)

1. Clinical safety review by telephone call (or in person if same day as scheduled SoC craniotomy or other planned visit) to assess concomitant medications and AEs.
2. Record any changes in concomitant medications since Screening (Visit 1).
3. Record any AEs which have occurred during the safety reporting period (from the time of ^{18}F-fluciclovine administration until 1 day post ^{18}F-fluciclovine administration). For scheduling purposes, the subject may be contacted up to 3 days after IMP injection, but the assessment is to be limited to the first day after IMP injection only.

10.5. Visit 4 – SoC Craniotomy (Pre-planned, 1 to 21 days after IMP Injection/PET)

Visit 4 – SoC Craniotomy should also take place after both the Visit 2 on-study MRI brain scan and Visit 3 Safety Follow-up.

1. Document neurosurgical procedure:
   a. Record sites of resection of reference lesion, and if applicable, MRI surgical lesions pre-planned for resection.

      If SoC resection of the reference lesion and/or MRI surgical lesions could not be performed during the procedure, record the contributing clinical factors, and whether a SoC biopsy was performed in lieu

   b. Record sites of 'additional PET lesion(s)' which underwent biopsy/resection.*
   c. If applicable, record neuronavigation system used for neurosurgical procedure.
   d. Where resection was performed, upload image/screenshot of neuronavigation system depicting the surgical resection plan/approach (if neuronavigation was not used for resection, a representative screenshot of the post-procedure MRI demonstrating the resection cavity is suitable). Where biopsy was performed, upload image/screenshot of neuronavigation system depicting stereotactic coordinates of biopsy.
   e. Upload neurosurgical procedural report.
   f. Record any clinical factors associated with the neurosurgical intervention which impacted the type of procedure performed
2. Label specimens with lesion ID as referenced by the imaging core lab study imaging platform on the previously annotated scans.
3. Process specimens per SoC and local laboratory standard practice.
4. Ship a representative set of specimens to the central laboratory per the Sample Processing Manual.

5. Record results of local histopathology analysis.

*Undertaking biopsy/resection of additional PET lesion(s) identified on the ^{18}F-fluciclovine PET scan is at the discretion of on-site investigators. The lesions should be judged suggestive of brain metastasis, warranting confirmation according to SoC practice, be neurosurgically feasible and accordingly, planned for biopsy/resection. The subject will receive post-procedural management per institutional SoC.

10.6. Early Withdrawal

Early withdrawal is defined as discontinuing from study without having SoC craniotomy/surgery on or before Day 22.

- Record any AEs or changes to concomitant medication occurring during safety reporting period.
- Record reason for early withdrawal.

11. IMAGING PROTOCOL

Full details on the imaging protocol are in the Image Acquisition Standards (PET scan imaging and processing only).

11.1. ^{18}F-fluciclovine PET Scan Patient Preparation

Patients should be advised not to eat for at least 4 hours prior to administration of ^{18}F-fluciclovine injection. Patients may have water or other clear fluids within this time window.

11.2. PET/CT Scanner

A dedicated hybrid PET scanner (e.g., PET/CT, PET/MRI) is mandatory. The selected PET scanner must be qualified by the study management team.

11.3. ^{18}F-fluciclovine Injection Administration

See Section 8.8 and Pharmacy Manual for administration instructions.

11.4. ^{18}F-fluciclovine PET Acquisition

For the dynamic PET acquisition, patients will be imaged for approximately 30 minutes. For the CT acquisition (if acquired on a PET/CT scanner), an unenhanced (no IV contrast) CT will be employed.

11.5. On-Study MRI Acquisition

All subjects will receive an on-study, study-specific brain MRI scan consisting of standard MRI sequences including (1) T_1-weighted MRI without and with contrast enhancement, (2) FLAIR and/or T_2-weighted MRI, and (3) diffusion-weighted imaging, ideally on the same day as the PET scan or up to a maximum of 3 days after the PET scan.

11.6. Image Transfer

Following the completion of PET and on-study MRI imaging at the study site, the scan data will be sent to the Imaging Core Lab using either the Imaging Management Solution software or on physical media by courier.

11.7. Image Evaluability

Both PET and on-study MRI scans will be assessed by the Imaging Core Lab for evaluability based on the Image Quality Standards. All data will undergo quality control including technical analysis, indication and protocol specific criteria, based on parameters detailed in the Image Acquisition Standards.

11.8. Central Image Analysis

Central image analysis of ^{18}F-fluciclovine PET, with Visit 2 on-study MRI used for anatomical reference, will be performed by 3 independent readers blinded to all clinical information.

1. Central image analysis of Visit 2 PET, with Visit 2 on-study MRI used for anatomical reference (i.e. using the on-study MRI to show the anatomical location of the

'reference lesion', 'MRI surgical lesion(s)' and where applicable 'additional PET lesion(s)')

Qualitative analysis: 3 independent reader evaluation, with Visit 2 on-study MRI used for anatomical reference.

- ^{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.
- 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 5 s, 6 frames of 10 s, 6 frames of 30 s and 5 frames of 60 s; from 10 min to 30 min after injection – 5 minute acquisition frames (Law, 2018).
  - 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

Quantitative analysis

- Lesion(s) and background structures will be outlined visually on static imaging data (e.g., from 10-20 minutes post-injection). This will be performed by the same 3 independent readers performing the qualitative analysis.
- Perform quantitative measure of background activity: mean standardized uptake value (SUV_{mean}).
- Perform quantitative measures of lesion ^{18}F-fluciclovine uptake, including: maximum standardized uptake value (SUV_{max}), peak standardized uptake value (SUV_{peak}), maximum tumor: background ratio (TBR_{max}; tumor SUV_{max}: SUV_{mean} background), peak tumor: background ratio (TBR_{peak}; tumor SUV_{peak}, background SUV_{mean}).

2. Central histopathology
   - Specimens will be centrally reviewed. Detailed specification of this workflow, and assignment of recurrent metastasis (positive) vs no recurrent metastasis (negative) based on the central review, will be laid out prospectively in a histopathology charter.

3. Image interpretation criteria
    - Central image analysis and central histopathologic correlate results will be reviewed by a separate Image Interpretation Committee (including representatives from the Sponsor and expert readers) to determine a set of image interpretation criteria based on the described visual reads, quantitative and dynamic measures.

Additional details on the central image analysis and Image Interpretation Committee will be provided in the Image Interpretation Standards and Image Interpretation Committee Plan, respectively.

12. ADVERSE EVENT REPORTING AND DOCUMENTATION

12.1. 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.

12.2. AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 5 (November 27, 2017) will be used to assess and grade event severity, including laboratory abnormalities judged to be clinically significant. For events not specifically mentioned in CTCAE, the general severity grading is provided below (Table 2).

Table 2. CTCAE (V5) AE Severity Grading

Severity (Toxicity Grade)	Description
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL.*
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE

ADL, activities of daily living; CTCAE, Common Terminology Criteria for Adverse Events.
*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
** Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

12.3. Adverse Event Relationship to IMP

The relationship of an AE to IMP will be categorized as follows:

Related: when there is a reasonable possibility of a causal relationship between IMP administration and an AE (i.e., adverse drug reaction [ADR])

Not related: when an AE does not follow a reasonable temporal sequence from IMP administration or when an AE can be reasonably explained by other factors including underlying disease, concomitant drugs or concurrent treatment.

12.4. Serious Adverse Event (SAE)

An SAE is defined as any adverse event occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed above.

12.4.1. Serious Adverse Event Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) on an SAE Report Form. The collection period for all SAEs will begin after initiation of ^{18}F-fluciclovine injection and end at Day 2. After this point, the investigator is not required to actively solicit SAE information from subjects. However, if they should become aware of an SAE that they suspect is related to the IMP, then they should report it. Any unresolved SAE beyond Day 2 at Visit 3 safety follow-up phone call, will be followed until resolution or stabilization.

All SAEs must be reported within 24 hours of the site study team becoming aware of the event by sending the completed SAE Report Form by fax, or scanned and emailed, to:

Bracco Diagnostics Inc. Drug Safety Unit:
drugsafetyus@blueearthdx.com
Fax: +1 609-514-2522

Additional and further requested information (follow-up or corrections to the original event) will be detailed on a new SAE Report Form and faxed/emailed to the same address.

For the US, in accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC) the site investigator will report SAEs to the IRB/IEC.

12.4.2. Suspected Unexpected Serious Adverse Reactions (SUSAR)

A SUSAR is a serious adverse reaction, the nature and severity of which is not consistent with the reference safety information set out in the Investigator's Brochure.

The Sponsor will be responsible for reporting all SUSARs to the relevant authorities, and other parties, as applicable.

Investigators will be informed of all SUSARs for the relevant IMP for all studies sponsored by Blue Earth Diagnostics, whether or not the event occurred in the current study.

12.4.3. Pregnancy

In the unlikely event of a pregnancy arising from sexual conduct performed within 24 hours following IMP administration, it will require expedited reporting to the sponsor's pharmacovigilance department within the same timelines as an SAE. Women who become pregnant between the Screening visit and Visit 2 should be withdrawn from the study. All reported pregnancies in study participants should be followed and the outcome reported using the same form. If the outcome of the pregnancy meets any of the criteria for seriousness, it must also be reported as an SAE. Examples of pregnancy outcomes that are SAEs:

- Reports of congenital anomalies or developmental delay, in the fetus or the child.
- Reports of fetal death and spontaneous abortion.
- Reports of suspected adverse reactions in the neonate that are classified as serious.

13. DISCONTINUATION AND REPLACEMENT OF SUBJECTS

13.1. Discontinuation/Withdrawal from the Study

Subjects are free to withdraw from participation in the study at any time, for any reason, specified or unspecified, and without prejudice.

An investigator may discontinue or withdraw a subject for the following reasons:

- Significant protocol violation or non-compliance
- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interests of the subject (investigator decision)
- Protocol violation requiring discontinuation of study treatment
- If the subject meets the exclusion criteria (either newly developed or not previously recognized) that precludes further study participation
- Lost to follow-up
- Sponsor request for early termination of study
- Subject requests to be withdrawn from the study
- Positive pregnancy test (females).

The reason for subject discontinuation or withdrawal from the study, if known, will be recorded in the subject's source documents and in the eCRF.

Refer to Section 10.6 for early withdrawal procedures.

13.2. Replacement of Subjects

Subjects who sign the informed consent form (ICF) and do not receive the study scan may be replaced. Subjects who sign the ICF and receive the study scan, but the scan is not considered evaluable (refer to Section 11 of the Imaging Protocol), may be replaced. Subjects who sign the ICF, and receive the study scan, and subsequently withdraw, or are withdrawn or discontinued from the study, prior to completed reference lesion resection at craniotomy/surgery, may be replaced.

13.3. Lost to Follow-up

A subject will be considered lost to follow-up if he or she fails to complete the Visit 3 post-IMP safety follow-up phone call or fails to continue through to Visit 4/Day 2-22 for SoC craniotomy/surgery and is unable to be contacted by the study site staff.

The following actions must be taken if a subject fails to complete a required study visit:

- The site will attempt to contact the subject and reschedule the missed visit prior to pre-scheduled Visit 4/Day 2-22 SoC craniotomy/surgery and ascertain if the subject wishes to and/or should continue in the study
- Before a subject is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address

or local equivalent methods). These contact attempts should be documented in the subject's medical record or study file.

- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up. A subject will be considered unreachable when:
  - Has received IMP and is beyond 20 days post-IMP administration.
  - Has not received IMP and is beyond 28 days of pre-study SoC MRI.

14. PROTOCOL VIOLATIONS

A protocol violation occurs when the subject, investigator, or Sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication
- Use of a non-validated scanner or imaging technique

Significant non-compliance from Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Sponsor will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and reported to the Sponsor for further assessment of the violation and any potential impact on ongoing protocol requirements and/or on the utility of the subject's collected study data.

15. STATISTICAL METHODS AND CONSIDERATIONS

Prior to database lock and analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be prepared and approved, describing all analyses to be performed. The SAP will document any changes to the analyses described below. Continuous data will be summarized using mean, standard deviation, median, minimum and maximum. Categorical data will be summarized using frequency counts and percentages.

15.1. Estimation 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.

15.2. 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 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 evaluable Visit 2 on-study PET scan (see Section 11.7) , and an evaluable central histopathology report (defined in the Histopathology Charter) from the sample obtained from the Visit 4 SoC craniotomy.

15.3. Subject Disposition

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.

15.4. Demographic and Baseline Characteristics

Demographic variables (including age, sex, ethnicity and race) and baseline characteristics (e.g., height, weight, body mass index [to be calculated], ECOG performance status, medical history, prior treatment history) will be summarized using appropriate summary statistics for the FAS, SAF and IEAS.

15.5. Analysis of Primary Endpoint

The primary endpoint is the diagnostic performance (sensitivity, specificity, PPV and 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.

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

Estimates and the 95% exact confidence intervals for sensitivity, specificity, PPV and NPV for each threshold will be calculated using standard definitions:

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

- 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. Lesions annotated on the scans will be matched to the histopathology results based on the lesion identifiers assigned by the study imaging platform at time of annotation. The results of these analyses will be reviewed by the separate Image Interpretation Committee (consisting of representatives from the Sponsor, contracted imaging core lab, and expert readers) to determine image interpretation criteria based on visual reads.

15.6. Analysis of Secondary Endpoint(s)

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 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 using the standard definitions stated above.

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)

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.

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. Adverse event verbatim terms will be coded to preferred terms and system organ classes using the latest version of Medical Dictionary for Regulatory Activities (MedDRA). Severity of AEs will be graded using the NCI-CTCAE v5.0. The frequency of TEAEs will be summarized overall, by system organ class and preferred term, and by severity. IMP-related AEs and SAEs will also be summarized.

15.7. Planned Interim Analysis

Not applicable.

16. DATA COLLECTION, RETENTION AND MONITORING

16.1. Data Collection Instruments

The investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject administered the study drug. Particular care should be taken to ensure all data points are recorded in source documentation, especially those which are not part of standard practice.

The investigator will collect relevant pre- and post-treatment MRI brain scans at Screening and upload the MRI images to the central imaging core lab.

Documentation of subject cancer history must include:

- Type of primary tumor that gave rise to the brain metastasis
- Previous treatments for brain metastases
- Previous cancer treatments for primary tumor (and other previous cancers, if applicable)
- Histopathological confirmation of the primary tumor or a metastatic site within 4 years of Screening.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific eCRF when the information corresponding to that visit is available.

Subjects will not be identified by name in the study database or on any study documents to be collected by the Sponsor, but will be identified by a site number and subject number.

If a correction is required for an eCRF, the time and date stamps track the person entering or updating eCRF data and creates an electronic audit trail.

The investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the investigator.

16.2. Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

16.3. Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Queries are entered, tracked, and resolved through the electronic data capture (EDC) system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

16.4. Archiving of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

16.5. Availability and Retention of Investigational Records

The investigator should maintain adequate and accurate source documents and study records that include all pertinent observations on each of the site's study subjects. Source data should be attributable, legible, contemporaneous, original, accurate and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary.

The investigator / institution should maintain the study documents as specified in Section 8 of International Council for Harmonisation (ICH) GCP E6 (R2) and as required by the applicable regulatory requirement(s). The investigator should take measures to prevent accidental or premature destruction of these documents.

Upon request of the monitor, auditor, IRB/IEC, or regulatory authority, the investigator must make available for direct access all requested study-related records.

Study records should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention / at least 25 years after the end of the clinical trial. These documents should be retained for a longer period, however, if required by applicable local regulations. No records will be destroyed without the written consent of the Sponsor, if applicable. It is the responsibility of the Sponsor to inform the investigator when these documents no longer need to be retained.

The medical files of subjects shall be archived in accordance with applicable local regulations.

16.6. Monitoring and Auditing

Monitoring visits will be conducted by representatives of the Sponsor according to ICH GCP and relevant regulations. By signing this protocol, the investigator grants permission to the Sponsor's (or designee's) monitors and auditors, as well as the IRB/IEC and regulatory authorities to conduct on-site monitoring and/or auditing and provide direct access to all requested study-related records.

16.7. Subject Confidentiality

In order to maintain subject confidentiality, only a site number and subject number will identify all study subjects on eCRFs and other documentation submitted to the Sponsor. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

Clinical information will not be released without written permission of the subject, except as necessary for monitoring by Regulatory Authorities. The investigator must also comply with

all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, etc.).

17. REGULATORY, ETHICAL AND STUDY OVERSIGHT CONSIDERATIONS

The study will be conducted in accordance with ICH GCP and all applicable regulations. The investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Sponsor and documented approval from the relevant regulatory authority (if applicable) and IRB/IEC, except where necessary to eliminate an immediate hazard(s) to the trial subjects. All personnel involved in the conduct of this study have completed ICH GCP training, relevant to their role.

17.1. Institutional Review Boards and Independent Ethics Committees

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning subject recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC.

The IRB/IECs written unconditional approval / favorable opinion of the study, and any additional local approvals (e.g., hospital management, Radiation Safety Committee, etc.), must be obtained prior to shipment of study drug to the site and prior to any subjects undergoing study-specific procedures. The investigator will obtain assurance of IRB/IEC compliance with regulations. Note: Regulatory authority approvals may also be required.

The IRB/IEC's standard operating procedures and policies will be followed for the submission of SAEs and progress reports during the conduct of the study.

An end of study notification will be submitted per regulatory requirements.

17.2. Amendments

Any decision to amend the clinical trial application and / or associated documents (e.g., protocol, informed consent form, CRF, Investigator's Brochure, etc.) will be made by the Sponsor.

The relevant regulations will be followed to determine what approvals from regulatory, IRB/IEC or local bodies are required. All required approvals will be obtained prior to implementation of the amendment, except as necessary to eliminate immediate safety hazards to subjects in accordance with ICH E6 (R2), 4.5 and applicable regulatory requirements. The Sponsor will notify each participating investigator site when the amendment can be implemented.

All changes to the consent form will be IRB/IEC approved; a determination will be made regarding whether a new consent needs to be obtained from subjects who provided consent, using a previously approved consent form.

17.3. Subject Information and Consent/Assent

In obtaining and documenting subject informed consent, the investigator must comply with the application regulatory requirement(s), ICH GCP and the ethical principles that have their origin in the Declaration of Helsinki.

Subject information and consent forms, and any other written material provided to the subject, must be approved by the relevant IRB/IEC (and by any other body as required by national regulations) prior to the start of the study at each study site.

The investigator (or an appropriately qualified designee) will explain the study to the subject or, if the subject is unable to provide informed consent, the subject's LAR, and answer any questions that arise. A verbal explanation will be provided in terms suited to the subject's, or subject's LAR's, comprehension, of the purposes, procedures, and potential risks of the study and the rights of research subjects. Subjects (and the subject's LAR) will have the opportunity to carefully review the written information and consent form, to discuss the study with their family or surrogates, and be given ample time to think about the study and ask questions before agreeing to participate.

Subjects (and the subject's LAR) must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Prior to the subject undergoing any study-specific procedures, the written informed consent form must be signed and personally dated by the subject, or their LAR, and by the person who conducted the informed consent discussion. The informed consent process will also be documented in the source document (including the date/time consent was obtained).

If a subject is unable to read or if a LAR is unable to read, an impartial witness should be present during the entire informed consent discussion. The subject, or subject's LAR, may orally consent to the subject's participation, if the subject, or subject's LAR is not capable of providing of signing and personally dating the consent form. Once the subject, or subject's LAR, has provided consent, the witness should also sign and personally date the consent form. By signing the consent form the witness attests that the information sheet / consent form was accurately explained to, and apparently understood by, the subject, or subject's LAR, and that informed consent was freely given by the subject, or subject's LAR.

The distribution of the signed information sheet /consent form will be as required by any applicable local regulations. Otherwise a copy of the signed informed consent document will be given to the subject and the original maintained with the subject's records.

The subject or subject's LAR, will be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the study. The communication of this information will be documented in the source documentation. The written subject information/consent form and any other written information provided to the subjects should be revised whenever important new information becomes available that may be relevant to the subject's consent. Any revised written subject information and consent form should receive IRB/IEC approval / favorable opinion prior to use. The subject, or subject's LAR, should sign and personally date any revised consent form and receive a copy (or original, if required by applicable regulations).

17.4. Post-trial Care

^{18}F-fluciclovine is a single-use diagnostic agent. No additional care for trial subjects is therefore planned once their participation through Visit 3 Safety Follow-Up has ended. All subjects will receive SoC treatment in-line with their medical condition as determined by their physician.

17.5. Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the

study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996 and the General Data Protection Regulation (EU) 2016/679.

17.6. Investigator Responsibilities

By signing the Protocol Agreement form, the investigator agrees to:

1. Comply with ICH GCP principles and all applicable regulatory requirements; be familiar with the appropriate use of the investigational product(s) as described in the protocol, Investigator's Brochure, and any other information sources provided.
2. Personally conduct or supervise the study; maintain a list of appropriately qualified persons to whom significant trial-related duties are delegated. Ensure all persons assisting with the study are adequately informed about the protocol, the investigational product(s), and their study-related duties.
3. Ensure that all study-related medical decisions are made by a qualified physician who is an investigator or sub-investigator for the study; ensure that adequate medical care is provided to a subject for any adverse events.
4. Obtain the written approval / favorable opinion of the IRB/IEC before the study starts; provide the IRB/IEC with the current Investigator's Brochure and all documents subject to review through-out the trial.
5. Submit a written progress report at least annually and in accordance with the IRB/IEC's request; submit an end of trial notification / final report to the IRB/IEC at the end of the study.
6. Conduct the study in compliance with the approved protocol and not implement any deviation from, or changes to the protocol without the agreement of the Sponsor, and prior approval / favorable opinion of the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard to a study subject, or when the change(s) involve only logistical or administrative aspects of the trial.
    a. Promptly (immediately) notify the Sponsor and IRB/IEC of any actions taken to eliminate an immediate hazard to a study subject in accordance with ICH E6 (R2), 4.5 and applicable regulatory requirements.
    b. Comply with ICH GCP, applicable regulatory requirements and the ethical principles that have their origin in the Declaration of Helsinki, when obtaining and documenting informed consent.
7. Ensure that investigational product(s) are: stored as specified by the Sponsor and in accordance with applicable regulatory requirement(s); used in accordance with the protocol and that adequate records are maintained.
8. Immediately report all SAEs to the Sponsor unless otherwise specified in the protocol or other document (e.g., Investigator's Brochure). Comply with applicable regulatory requirement(s) related to the reporting of unexpected serious adverse drug reactions to regulatory authorities and the IRB/IEC.
9. If the study is prematurely terminated or suspended for any reason, promptly inform study subjects and assure appropriate therapy and follow-up, as required; follow ICH GCP and required regulatory requirements to notify the Sponsor and IRB/IEC.

10. Maintain adequate and accurate source documents and study records that include all pertinent observations on each of the study subjects; source data should be attributable, legible, contemporaneous, original, accurate and complete. Changes to source data must be traceable, should not obscure the original entry and should be explained if necessary.
11. Retain essential documents for at least 2 years of a marketing application /at least 25 years or until notified by the Sponsor.
12. Provide monitors, auditors, IRB/IEC and regulatory authorities direct access to all requested study-related records.

18. REFERENCES

1. Arvold ND, Lee EQ, Mehta MP, et al. Updates in the management of brain metastases. Neuro Oncol. 2016;18(8):1043-65. (Arvold, 2016)
2. Bach-Gansmo T, Nanni C, Nieh PT, et al. Multisite experience of the safety, detection rate and diagnostic performance of Fluciclovine (18F) positron emission tomography/computerized tomography imaging in the staging of biochemically recurrent prostate cancer. J Urol. 2017;197(3):676-83. (Bach-Gansmo, 2017)
3. Bogsrud TV, Londalen A, Brandal P, Leske H, Panagopoulos I, Borghammer P, et al. ^{18}F-Fluciclovine PET/CT in suspected residual or recurrent high-grade glioma. Clin Nucl Med. 2019. (Bogsrud, 2019)
4. Brown C, Dempsey MF, Gillen G, et al. Investigation of 18F-FDG 3D mode PET image quality versus acquisition time. Nuc Med Commun. 2010;31 (3):254-9. (Brown, 2010)
5. Ceccon G, Lohmann P, Stoffels G, Judov N, Filss CP, Rapp M, et al. Dynamic O-(2-18F-fluoroethyl)-L-tyrosine positron emission tomography differentiates brain metastasis recurrence from radiation injury after radiotherapy. Neuro Oncol. 2017;19(2):281-8. (Ceccon, 2017)
6. Cicone F, Minniti G, Romano A, Papa A, Scaringi C, Tavanti F, et al. Accuracy of F-DOPA PET and perfusion-MRI for differentiating radionecrotic from progressive brain metastases after radiosurgery. Eur J Nucl Med Mol Imaging. 2015;42(1):103-11. (Cicone, 2015)
7. Dequesada IM, Quisling RG, Yachnis A, Friedman WA. Can standard magnetic resonance imaging reliably distinguish recurrent tumor from radiation necrosis after radiosurgery for brain metastases? A radiographic-pathological study. Neurosurgery. 2008;63(5):898-903; discussion 4. (Dequesada, 2008)
8. FDA/NBTS. FDA-NBTS Public Workshop: product development for central nervous system (CNS) metastases. Available at: https://www.fda.gov/drugs/news-events-human-drugs/fda-nbts-public-workshop-product-development-central-nervous-system-cns-metastases [Accessed: 31 July 2019]. 2019. (FDA/NBTS, 2019)
9. Fuchs BC, Bode BP. Amino acid transporters ASCT2 and LATl in cancer: partners in crime? Semin Cancer Biol. 2005;15(4):254-66. (Fuchs, 2005)
10. Galldiks N, Stoffels G, Filss CP, Piroth MD, Sabel M, Ruge MI, et al. Role of O-(2-(18)F-fluoroethyl)-L-tyrosine PET for differentiation of local recurrent brain metastasis from radiation necrosis. J Nucl Med. 2012;53(9):1367-74. (Galldiks, 2012)
11. Galldiks N, Langen KJ, Albert NL, et al. PET imaging in patients with brain metastasis-report of the RANO/PET group. Neuro Oncol. 2019;21(5):585-95. (Galldiks, 2019)
12. Heiss WD. Clinical impact of amino acid PET in gliomas. J Nucl Med. 2014;55(8):1219-20. (Heiss, 2014)
13. Henderson F, Brem S, O'Rourke DM, Nasrallah M, Buch VP, Young AJ, et al. ^{18}F-Fluciclovine PET to distinguish treatment-related effects from disease progression in recurrent glioblastoma: PET fusion with MRI guides neurosurgical sampling. Neuro-Oncology Practice. 2019:1-6. (Henderson, 2019)

14. Kann BH, Park HS, Johnson SB, et al. Radiosurgery for brain metastases: changing practice patterns and disparities in the United States. J Natl Compr Cane Netw. 2017; 15(12): 1494-502. (Kann, 2017)

15. Karlberg A, Berntsen EM, Johansen H, Skjulsvik AJ, Reinertsen I, Dai HY, et al. 18F-FACBC PET/MRI in diagnostic assessment and neurosurgery of gliomas. Clin Nucl Med. 2019;44(7):550-9. (Karlberg, 2019)

16. Kaufmann TJ, Smits M, Boxerman J, Huang R, Barboriak DP, Weller M, et al. Consensus recommendations for a standardized brain tumor imaging protocol for clinical trials in brain metastases. Neuro Oncol. 2020; noaa030, https://doi.org/10.1093/neuonc/noaa030. (Kaufmann, 2020)

17. Kocher M, Soffietti R, Abacioglu U, Villa S, Fauchon F, Baumert BG, et al. Adjuvant whole-brain radiotherapy versus observation after radiosurgery or surgical resection of one to three cerebral metastases: results of the EORTC 22952-26001 study. J Clin Oncol. 2011;29(2):134-41. (Kocher, 2011)

18. Kondo A, Ishii H, Aoki S, et al. Phase Ila clinical study of [18F]fluciclovine: efficacy and safety of a new PET tracer for brain tumors. Ann Nucl Med. 2016;30(9):608-18. (Kondo, 2016)

19. Langen KJ, Galldiks N. Update on amino acid PET of brain tumours. Curr Opin Neurol. 2018;31(4):354-61. (Langen, 2018)

20. Langen KJ, Galldiks N, Hattingen E, Shah NJ. Advances in neuro-oncology imaging. Nat Rev Neurol. 2017;13(5):279-89. (Langen, 2017)

21. Law I, Albert NL, Arbizu J, Boellaard R, Drzezga A, Galldiks N, et al. Joint EANM/EANO/RANO practice guidelines/SNMMI procedure standards for imaging of gliomas using PET with radiolabelled amino acids and [18F]FDG: version 1.0. Eur J Nucl Med Mol Imaging. 2019;46(3):540-57. (Law, 2018)

22. Lin NU, Lee EQ, Aoyama H, Barani IJ, Barboriak DP, Baumert BG, et al. Response assessment criteria for brain metastases: proposal from the RANO group. Lancet Oncol. 2015;16(6):e270-8. (Lin, 2015)

23. Lizarraga KJ, Allen-Auerbach M, Czernin J, DeSalles AA, Yong WH, Phelps ME, et al. (18)F-FDOPA PET for differentiating recurrent or progressive brain metastatic tumors from late or delayed radiation injury after radiation treatment. J Nucl Med. 2014;55(1):30-6. (Lizarraga, 2014)

24. McParland BJ, Wall A, Johansson S, et al. The clinical safety, biodistribution and internal radiation dosimetry of [18F]fluciclovine in healthy adult volunteers. Eur J Nucl Med Mol Imaging. 2013;40(8): 1256-64. (McParland, 2013)

25. Michaud L, Beattie BJ, Akhurst T, Dunphy M, Zanzonico P, Finn R, et al. (18)F-Fluciclovine ((18)F-FACBC) PET imaging of recurrent brain tumors. Eur J Nucl Med Mol Imaging. 2020;47: 1353-67. (Michaud, 2020)

26. Minamimoto R, Saginoya T, Kondo C, Tomura N, Ito K, Matsuo Y, et al. Differentiation of brain tumor recurrence from post-radiotherapy necrosis with 11C-methionine PET: visual assessment versus quantitative assessment. PLoS One. 2015;10(7):e0132515. (Minamimoto, 2015)

27. NCCN 2019. NCCN Guidelines® Central Nervous System Cancers (5 March 2019). Available at: https://www.nccn.org/professionals/physician gls/default.aspx [Accessed: 31 July 2019]. (NCCN, 2019)

28. Oka S, Okudaira H, Yoshida Y, Schuster DM, Goodman MM, Shirakami Y. Transport mechanisms of trans-1-amino-3-fluoro[1-(14)C]cyclobutanecarboxylic acid in prostate cancer cells. Nucl Med Biol. 2012;39(1):109-19. (Oka, 2012)

29. Oka S, Okudaira H, Ono M, Schuster DM, Goodman MM, Kawai K, et al. Differences in transport mechanisms of trans-1-amino-3-[18F]fluorocyclobutanecarboxylic acid in inflammation, prostate cancer, and glioma cells: comparison with L-[methyl-11C]methionine and 2-deoxy-2-[18F]fluoro-D-glucose. Mol Imaging Biol. 2014;16(3):322-9. (Oka, 2014)

30. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982;5:649-55. (Oken, 1982)

31. Okudaira H, Shikano N, Nishii R, Miyagi T, Yoshimoto M, Kobayashi M, et al. Putative transport mechanism and intracellular fate of trans-1-amino-3-18F-fluorocyclobutanecarboxylic acid in human prostate cancer. J Nucl Med. 2011;52(5):822-9. (Okudaira, 2011)

32. Ono M, Oka S, Okudaira H, Schuster DM, Goodman MM, Kawai K, et al. Comparative evaluation of transport mechanisms of trans-1-amino-3-[18F]fluorocyclobutanecarboxylic acid and L-[methyl-11C]methionine in human glioma cell lines. Brain Res. 2013;1535:24-37. (Ono, 2013)

33. Parent EE, Benayoun M, Ibeanu I, Olson JJ, Hadjipanayis CG, Brat DJ, et al. ^{18}F-Fluciclovine PET discrimination between high- and low-grade gliomas. EJNMMI Res. 2018;8(1):67. (Parent, 2018)

34. Patel D, Nye J, Schuster D, et al. Fluciclovine PET imaging of intracranial metastases. J Nucl Med 2018;59(suppl 1): Abstract 1097 and Conference Poster. (Patel, 2018)

35. Peng L, Parekh V, Huang P, Lin DD, Sheikh K, Baker B, et al. Distinguishing true progression from radionecrosis after stereotactic radiation therapy for brain metastases with machine learning and radiomics. Int J Radiat Oncol Biol Phys. 2018;102(4):1236-43. (Peng, 2018)

36. Pope WB. Brain metastases: neuroimaging. Handb Clin Neurol. 2018;149:89-112. (Pope, 2018)

37. Romagna A, Unterrainer M, Schmid-Tannwald C, Brendel M, Tonn JC, Nachbichler SB, et al. Suspected recurrence of brain metastases after focused high dose radiotherapy: can ^{18}F-FET- PET overcome diagnostic uncertainties? Radiat Oncol. 2016;11(1):139. (Romagna, 2016)

38. Schuster DM, Votaw JR, Halkar RK, et al. Uptake of the synthetic PET amino acid radiotracer 1-amino-3-l 8F-fluorocyclobutane-1-carboxylic acid (18F-FACBC) within primary and metastatic brain cancer compared with ^{18}F-fluorodeoxyglucose (^{18}F-FDG). J Nucl Med. 2003;44(suppl): Abstract 167P. (Schuster, 2003)

39. Schuster DM, Nanni C, Fanti S, et al. Anti-1-amino-3-18F-fluorocyclobutane-1-carboxylic acid: physiologic uptake patterns, incidental findings, and variants that may simulate disease. J Nuc Med 2014;55:1986-92. (Schuster, 2014)

40. Sinha R, Sage W, Watts C. The evolving clinical management of cerebral metastases. Eur J Surg Oncol. 2017;43(7):1173-85. (Sinha, 2017)

41. Stockham AL, Tievsky AL, Koyfman SA, Reddy CA, Suh JH, Vogelbaum MA, et al. Conventional MRI does not reliably distinguish radiation necrosis from tumor recurrence after stereotactic radiosurgery. J Neurooncol. 2012;109(1):149-58. (Stockham, 2012)

42. Suh JH. Stereotactic radiosurgery for the management of brain metastases. N Engl J Med. 2010;362(12):1119-27. (Suh, 2010)

43. Teoh E, Morotti M, Bridges E, Jones D, Miar A, Wigfield S, et al. Fluciclovine (18F) is a marker for high-affinity glutamine transporter ASCT2-mediated amino acid transport in breast cancer. J Nucl Med. 2017;58(suppl 1):1028. (Teoh, 2017)

44. Terakawa Y, Tsuyuguchi N, Iwai Y, Yamanaka K, Higashiyama S, Takami T, et al. Diagnostic accuracy of 11C-methionine PET for differentiation of recurrent brain tumors from radiation necrosis after radiotherapy. J Nucl Med. 2008;49(5):694-9. (Terakawa, 2008)

45. Tsuyuguchi N, Terakawa Y, Uda T, Nakajo K, Kanemura Y. Diagnosis of brain tumors using amino acid transport PET imaging with (18)F-fluciclovine: A comparative study with L-methyl-(11)C-methionine PET imaging. Asia Ocean J Nucl Med Biol. 2017;5(2):85-94. (Tsuyuguchi, 2017)

46. Vellayappan B, Tan CL, Yong C, Khor LK, Koh WY, Yeo TT, et al. Diagnosis and management of radiation necrosis in patients with brain metastases. Front Oncol. 2018;8:395. (Vellayappan, 2018)

47. Verma N, Cowperthwaite MC, Burnett MG, et al. Differentiating tumor recurrence from treatment necrosis: a review of neuro-oncologic imaging strategies. Neuro Oncol. 2013;15(5):515-34. (Verma, 2013)

48. Wakabayashi T, Iuchi T, Tsuyuguchi N, et al. Diagnostic performance and safety of positron emission tomography Using ^{18}F-Fluciclovine in patients with clinically suspected high- or low-grade gliomas: A multicenter phase IIb trial. Asia Ocean J Nucl Med Biol. 2017;5(1): 10-21. (Wakabayashi, 2017)

49. Walker AJ, Ruzevick J, Malayeri AA, Rigamonti D, Lim M, Redmond KJ, et al. Postradiation imaging changes in the CNS: how can we differentiate between treatment effect and disease progression? Future Oncol. 2014;10(7):1277-97. (Walker, 2014)

50. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. J Clin Oncol. 2010;28(11):1963-72. (Wen, 2010)

51. Zhang J, Liu H, Tong H, et al. Clinical applications of contrast-enhanced perfusion MRI techniques in gliomas: recent advances and current challenges. Contrast Media Mol Imaging. 2017;2017:7064120. (Zhang, 2017)

APPENDIX 1. ECOG PERFORMANCE STATUS

ECOG PERFORMANCE STATUS [a]	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead

ECOG = Eastern Cooperative Oncology Group.
[a] As published in Oken, 1982.

APPENDIX 2. PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include providing Blue Earth Diagnostics with the information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles, applicable regulatory requirements, and to abide by the terms of this protocol.

Protocol Number: 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)

Protocol Version: 3.0 **Protocol Date:** 09 June 2021

_____ _____

Investigator Signature Date

_____

Print Name and Title

Site # _____

Site Name _____

Address _____

_____

_____

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Phone Number _____

Email _____