

**Title:**

**[<sup>18</sup>F]Fluciclovine and [<sup>18</sup>F]FLT PET/CT Assessment of Primary High-Grade Brain Tumors**

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Imaging Agents to be used:

[<sup>18</sup>F] anti-FACBC ([<sup>18</sup>F]Fluciclovine)  
IND # - 136875 (Yap)

3'-deoxy-3'-[<sup>18</sup>F]fluorothymidine ([<sup>18</sup>F]FLT)  
IND # - 76,843 (Yap)

University of Utah IRB # 104704

**Historical Protocol Versions**

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## **PROTOCOL SIGNATURE**

I confirm that I have read this protocol, and I will conduct the study as outlined herein and according to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable ICH guidelines for good clinical practice, and the applicable laws and regulations of the federal government. I will promptly submit the protocol to the IRB for review and approval. Once the protocol has been approved by the IRB, I understand that any modifications made during the course of the study must first be approved by the IRB prior to implementation except when such notification is made to remove an immediate hazard to the subject.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study treatment, the conduct of the study, and the obligations of confidentiality.

Note: This document is signed electronically through submission and approval by the Principal Investigator in the University of Utah IRB Electronic Research Integrity and Compliance Administration (ERICA) system.

## 1. OBJECTIVES

### 1.1. Primary Objectives

Positron emission tomography (PET) is a molecular imaging modality that can probe various aspects of tumor function and biology using a variety of radiolabeled imaging agents (“tracers”). Oncologic PET imaging has seen a dramatic rise in clinical utilization over the past decade for cancer detection, staging, and evaluating residual or recurrent disease following therapy. These clinical scans use the FDA approved PET radiopharmaceutical [<sup>18</sup>F]fluoro-2-deoxy-D-glucose (FDG), which accumulates in cells in proportion to GLUT transporter and hexokinase activity. FDG thus provides a measure of tissue glucose metabolism. On May 27, 2016 [<sup>18</sup>F] anti-FACBC ([<sup>18</sup>F]Fluciclovine) with the brand name of Axumin was approved for detection of recurrent prostate cancer (Axumin 2016). ([<sup>18</sup>F]Fluciclovine is a synthetic L-leucine analogue that is transported into cells but is not incorporated into proteins. Primary brain tumors have upregulated amino acid utilization and thus increased [<sup>18</sup>F]Fluciclovine uptake. Another PET tracer that has received significant attention in research is 3'-deoxy-3'-[<sup>18</sup>F]fluorothymidine ([<sup>18</sup>F]FLT). The uptake, retention/washout, and ultimate biodistribution of these tracers ([<sup>18</sup>F]Fluciclovine and [<sup>18</sup>F]FLT) are each related to different functional or molecular processes. As such, each can be used to probe a different aspect of tumor biology: [<sup>18</sup>F]FLT directly assesses tumor proliferation (Shields et al., 1998, Rasey et al., 2002, Schwartz et al., 2003, Chen et al., 2005, Choi et al., 2005, Jacobs et al., 2005) and [<sup>18</sup>F]Fluciclovine provides a measure of tumor growth related to amino acid transport and utilization (Shoup and Goodman, 1999, Akhurst et al., 2006, Meirelles et al., 2006, Oka et al., 2007, Yu 2009, Yu 2010, Oka et al., 2012).

Assessing response, particularly early response, to therapy in malignant glioma is a challenging problem. Typically the extent of MRI contrast enhancement in malignant gliomas has been used as an indicator of therapeutic response (Grant et al., 1997). Several response criteria have been developed for the assessment of malignant gliomas including the Macdonald criteria (Macdonald et al., 1990) and more recently the Response Assessment in Neuro-Oncology Working Group (RANO) criteria (Wen et al., 2010). The major modification proposed by the RANO guidelines was the assessment of increasing fluid-attenuated inversion recovery (FLAIR) signal intensity change as evidence of tumor progression. This was particularly helpful when antiangiogenic therapy became a treatment option for malignant brain tumors. The RANO criteria still have many issues making their implementation difficult particularly in clinical trials (Pope and Hessel, 2011). Another known problem with the current response criteria is the fact that there is often viable tumor extending beyond the enhancing zone. Biopsy studies have shown that viable tumor can extend beyond the margin of  $T_2$  signal change in glioblastomas (Lunsford et al., 1986, Watanabe et al., 1992) and can extend up to 2.5 cm beyond the area of  $T_2$  signal change. There are reports of tumor being identified in regions with a normal  $T_1$  signal in 16% of biopsies and a normal  $T_2$  signal in 4% of biopsies (Kelly et al., 1987).

To date there have been no published human studies assessing the use of [<sup>18</sup>F]Fluciclovine or [<sup>18</sup>F]FLT to assess response to chemoradiation in primary high-grade brain tumors. This study is therefore exploratory as it will (1) define the semi-quantitative change in [<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine uptake which is consistent with response based on standard brain tumor response criteria. (2) define the semi-quantitative change in [<sup>18</sup>F]FLT and/or [<sup>18</sup>F]Fluciclovine uptake which predicts time to progression and overall survival. The standard response assessment will utilize standard MRI techniques in patients with high-grade brain tumors receiving standard chemoradiation and (3) assess the ability of [<sup>18</sup>F]FLT or [<sup>18</sup>F]Fluciclovine alone or together to predict true progression from pseudoprogression in patients with this clinical dilemma. The objective is more clearly described below. By assessing a biologic process such as proliferation with [<sup>18</sup>F]FLT and amino acid uptake with [<sup>18</sup>F]Fluciclovine, we may be able to better define viable tumor and better assess response and overall survival to chemoradiation.

This exploratory study will be in 30 patients with suspected or pathologically confirmed high-grade primary brain tumors. Two different groups of patients will be able to enroll in the trial. The first group, Group A, will be for patients who have not yet received any therapy for their suspected or newly found brain tumors. Group B will enroll patients with pathologically proven malignant brain tumor (WHO Grade III or IV glial-based tumors) who have undergone chemoradiation and have a contrast enhancing mass  $\geq 1.0$  cm in greatest diameter concerning for pseudoprogression. MRI-documented possible recurrence/progression versus treatment effect (pseudoprogression) within 6 months from the time of completion of chemoradiation.

Group A will have three different patient cohorts. The first, for patients where there is compelling evidence, based on the MRI and/or CT imaging, that a high-grade primary brain tumor is present. Pathologic confirmation will occur with subsequent biopsy or surgery. The second cohort of patients are individuals where the tumor is felt to be inoperable and a biopsy is performed but no surgery. The third cohort of patients are post-surgery where a complete surgical resection was not possible. This will be confirmed by a post-operative contrast MRI/CT scan where residual tumor  $\geq 1.0$  cm in diameter is present.

The study will include PET imaging procedures at up to three possible time points: (1) at “baseline” prior to any tumor-directed chemoradiotherapy (2) At the conclusion of the initial chemoradiotherapy (1-4 weeks post last treatment); and (3) at any time of MRI-documented possible recurrence/progression ( $\geq 1.0$  cm in greatest diameter) versus treatment effect (pseudoprogression) within 6 months from the time of completion of chemoradiation. Also, patients who were not originally enrolled in the study for baseline and conclusion of therapy imaging will be eligible to enroll at the time of MRI-documented possible recurrence/progression versus treatment effect (pseudoprogression) if within 6 months from the time of completion of chemoradiation.

A number of quantitative and semi-quantitative imaging biomarkers for each tracer will be computed at each imaging time point, and the change in each biomarker between time-points will also be computed. These data will be compared with clinical endpoints (standard RANO criteria, survival, time to progression), and with tumor biologic information (histology, WHO grade, vascularity, MIB-1, VEGF, EGFR, p53, IDH status) in cases when tumor tissue becomes available from standard care. These preliminary and exploratory data will provide information regarding semi-quantitative changes in uptake consistent with standard response, the potential value of concurrent multiple PET biomarkers for predicting tumor behavior prior to the start of therapy, for improved prognostication, for more efficient and effective tumor surveillance, and/or for more appropriate assignment of patients to conventional, aggressive, or investigational therapies early in their clinical courses. This initial data will be important for design of subsequent clinical trials using [<sup>18</sup>F]Fluciclovine and/or [<sup>18</sup>F]FLT to assess response to chemoradiation in high-grade primary brain tumor.

## **1.2. The Hypotheses to be Tested and Objectives**

The hypothesis of this exploratory clinical trial in patients with high-grade a primary brain tumor who receive chemoradiation is that the PET imaging agents [<sup>18</sup>F]Fluciclovine and/or [<sup>18</sup>F]FLT will be a better predictor of tumor response than standard MRI based brain tumor response criteria. When used in conjunction, the two PET agents may be better able to predict tumor aggressiveness and thus overall survival than the use of individual-tracer PET biomarkers. This may eventually lead to improved assessment of response (including time to progression and overall survival) and differentiation of tumor recurrence/progression from treatment effect (pseudoprogression).

The primary endpoint of the study is that a 25% reduction in  $SUV_{max}$  or  $SUV_{mean}$  for each of the PET imaging tracers will be predictive of increased overall survival.

## Secondary Exploratory Objectives

Assess the association between overall survival and change in each of the following quantitative markers for [<sup>18</sup>F]Fluciclovine and [<sup>18</sup>F]FLT:  $K_1-k_4$ ,  $K_{net}$  and  $K^{trans}$ , from baseline to post therapy.

Assess the association between progression free survival and change in each of the following quantitative markers for [<sup>18</sup>F]Fluciclovine and [<sup>18</sup>F]FLT:  $K_1-k_4$ ,  $K_{net}$  and  $K^{trans}$ , from baseline to post therapy.

Assess the ability of [<sup>18</sup>F]FLT and/or [<sup>18</sup>F]Fluciclovine alone or together to predict true progression from pseudoprogression in patients with this clinical dilemma.

A 25% reduction in  $SUV_{max}$  or  $SUV_{mean}$  for each of the PET imaging tracers will be more predictive of overall survival than the current MRI based response criteria.

A 25% reduction in  $SUV_{max}$  or  $SUV_{mean}$  for each of the PET imaging tracers will be more predictive of time to progression than the current MRI based response criteria.

## 2. BACKGROUND

### 2.1. Malignant Brain Tumors

Despite significant advances in the understanding of brain tumor biology and genetics as well as improvements in surgical techniques, radiotherapy administration, and chemotherapy methods; most primary brain tumors remain incurable. Most primary brain tumors are highly infiltrative neoplasms, and are therefore unlikely to be cured by local treatments such as surgery, focal radiotherapy, radiosurgery, or brachytherapy.

The clinical presentation of a brain tumor is highly dependent on its location within the nervous system; but includes signs/symptoms that relate directly to compression and/or infiltration of specific functional areas of brain parenchyma, increased intracranial pressure, hydrocephalus, cerebral edema, and/or vascular complications from the tumor. The most common symptoms include headaches, cognitive deficits, nausea/vomiting, seizures, balance disturbances, and focal neurologic deficits. Once clinically suspected of having such a lesion, a patient usually undergoes an imaging procedure, typically a computed tomography (CT) scan or a magnetic resonance (MR) scan. Neurosurgical intervention, in the form of either a brain biopsy or craniotomy with resection, is the next step to obtain a tissue diagnosis and, if warranted, to decrease the tumor mass.

There are approximately 16,900 new cases of primary CNS tumors diagnosed in the United States each year. Numerous studies have documented an increase in the incidence of these tumors in the last decades (Sanson-Fisher et al., 2000). About 50% of these lesions are supratentorial high-grade gliomas. Anaplastic astrocytoma (AA) (WHO Grade III) and glioblastoma (GBM) (WHO Grade IV) are the most common glial primary brain tumors with at least 80% of malignant gliomas being GBMs (Brock et al., 1998, Ichimura et al., 1998). Patients with newly diagnosed malignant gliomas are usually treated with biopsy or surgical resection followed by radiation therapy and/or chemotherapy; depending on their functional status. Despite these interventions, the median survivals for newly diagnosed GBM and AA are 50 and 150 weeks, respectively. Chemotherapy has been shown to increase the proportion of long-term survivors from less than 5% to between 15-20 % (Treat et al., 2012). Once a patient has failed initial therapy, salvage therapy is difficult and most patients die of tumor progression within 4-12 months (Group, 2002).

## 2.2. Brain Tumor Imaging with Magnetic Resonance Imaging (MRI)

When considering a broader approach to the diagnosis and treatment of these tumors and other CNS lesions, the blood-brain barrier (BBB) is an important obstacle to the delivery of both therapeutic and diagnostic imaging agents. The BBB is a unique feature of CNS capillaries that results from a continuous layer of endothelial cells bound together with tight junctions that allow very little transcellular or pericellular transport of blood-borne molecules (Neuwelt, 1989, Banks et al., 1999). The blood-tumor barrier in both primary cerebral tumors and in cerebral metastatic disease is often highly permeable compared to the normal BBB (Long, 1979, Neuwelt, 1989, Groothuis et al., 1991). This disruption of the integrity of the BBB allows the contrast agents typically used in MRI and CT imaging to leak from the vascular space and accumulate in the perivascular and intercellular spaces in tumors, causing altered signal known as enhancement in both CT and MRI imaging. The most commonly performed study today to assess the integrity of the blood brain barrier is a T1 MRI study obtained post-injection of gadolinium DTPA. There are currently several commercially-available and FDA-approved gadolinium based MRI contrast agents marketed and licensed in the United States for brain imaging. These imaging agents are non-specific; and, since there is also loss of integrity of the BBB in radiation necrosis and treatment effect, MRI imaging cannot be used to unequivocally differentiate tumor recurrence from radiation necrosis/treatment effect (Chaskis et al., 2009, da Cruz et al., 2011, Caroline and Rosenthal, 2012). Newer MRI techniques to assess pseudoprogession have been tried (see below), however, these have not found widespread clinical use (Chu et al., 2013, Gahramanov et al., 2013, Park et al., 2015, Yun et al., 2015). Magnetic resonance spectroscopy (MRS) has also been used in an attempt to differentiate radiation necrosis/treatment effect from recurrent tumor; unfortunately, the results were less than impressive (da Cruz et al., 2011, Sawlani et al., 2012). Consequently, both national coverage requests to the Center for Medicare and Medicaid Services (CMS) to reimburse MRI spectroscopy in the past decade have been denied.

## 2.3 Brain Tumor Pseudoprogession

Treatment of high-grade brain tumors (GBM and AA) with chemoradiation (temozolomide/radiation) can cause changes in tumor vascular permeability which alter the pattern of gadolinium enhancement, mimicking tumor progression or recurrence. These temporary vascular permeability changes usually improve or stabilize on follow-up MRI and have been termed “pseudoprogession” (Hoffman et al., 1979, De Wit et al., 2004, Chamberlain et al., 2007, Brandes et al., 2008, Brandsma et al., 2008, Fine et al., 2008, Brandsma and van den Bent, 2009, Chaskis et al., 2009, Wen et al., 2010) to distinguish them from true tumor progression. The lack of a definitive imaging modality to distinguish these post-treatment radiographic imaging changes (PTRIC), including pseudoprogession and radiation necrosis/treatment effect, from true tumor progression presents a major unmet clinical need in the care of GBM patients (Wen et al., 2010). To date, no single imaging modality (including MRI, MRS, and PET) has been able to consistently and accurately distinguish between PTRIC from true tumor progression. At present, only direct examination of tumor histopathology can accurately and consistently discriminate true tumor progression from PTRIC. Furthermore, there is no clear understanding of the molecular, biochemical, and cellular mechanisms mediating PTRIC. This clinical trial will hopefully provide a better understanding of the mechanisms responsible for this clinical dilemma and for the development of a novel PET imaging approach to distinguish PTRIC from true tumor progression.

## 2.4. Overview of the PET Tracers [<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine

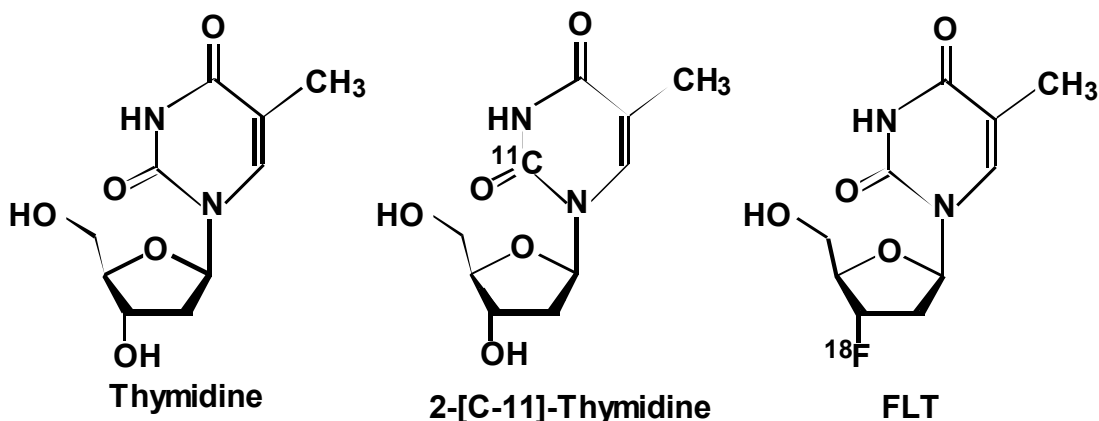
The use of more specifically targeted imaging agents, such as PET radiopharmaceuticals, has great potential for overcoming the limitations of MRI imaging in brain tumors



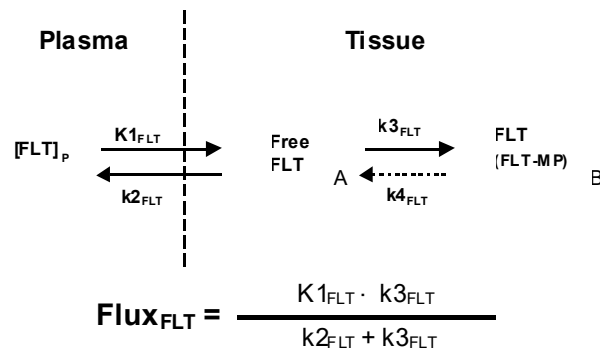
### 2.4.1. [<sup>18</sup>F]FLT as an Imaging Agent

3'-deoxy-3'-[<sup>18</sup>F]fluorothymidine: [<sup>18</sup>F]FLT (mol. wt. 244.2 for [F-19]FLT) is a structural analog of the DNA constituent, thymidine. [<sup>18</sup>F]FLT is a radiolabeled imaging agent that has been utilized for investigating cellular proliferation with PET (Shields et al., 1998, Rasey et al., 2002). Although [<sup>18</sup>F]FLT is not incorporated into DNA, it is trapped in the cell due to phosphorylation by thymidine kinase, a part of the proliferation pathway. As such it has the potential to image proliferating tumor in proportion to the DNA synthesis rate. In the case of the brain, [<sup>18</sup>F]FLT is not taken up or retained in normal brain tissue due to the intact blood-brain barrier as well as the paucity of proliferative activity. This gives [<sup>18</sup>F]FLT a distinct advantage compared to FDG as it should only be concentrated in viable proliferating tumor, allowing for improved differentiation of radiation necrosis from recurrence.

[<sup>18</sup>F]FLT has not been marketed in the United States and to the best of our knowledge, there has been no marketing experience with this drug in other countries. The radiopharmaceutical product, [<sup>18</sup>F]FLT is the only active ingredient and it is dissolved in a solution of  $\leq 10$  mL of 92% 0.01 M phosphate buffered saline (PBS): 8% ethanol (v:v). The drug solution is stored at room temperature in a sealed, sterile, pyrogen-free syringe with an expiration time of 8 hours. The injectable dose of [<sup>18</sup>F]FLT for this study will typically be  $\leq 0.1$  mCi/kg of fluorine-18 not to exceed 10.0 mCi, with a specific activity greater than 200 Ci/mmol at the time of injection. The three required IND qualification runs performed in the cyclotron facility at the Huntsman Cancer Institute (HCI) had specific activities of 5600 Ci/mmol, 5223 Ci/mmol, and 4659 Ci/mmol (end of synthesis). In the dose of [<sup>18</sup>F]FLT only a small fraction of the FLT molecules are radioactive. The amount of injected drug is  $\leq 12.2$   $\mu$ g ( $\leq 50$  nmol) of FLT. [<sup>18</sup>F]FLT is administered to subjects by intravenous injection of  $\leq 10$  mL. There is no evidence that non-radioactive and radioactive FLT molecules display different biochemical behavior.



FLT undergoes relatively little degradation after injection aside from production of the glucuronide in the liver [Shields 2005]. Thymidine phosphorylase in the blood does not degrade FLT. The mathematical model which can be used to describe the uptake of [<sup>18</sup>F]FLT is therefore relatively simple since the only metabolite in the plasma that needs to be measured is the glucuronide conjugate.



Retention of [<sup>18</sup>F]FLT is reflected in the flux constant, Flux<sub>FLT</sub> or K<sub>FLT</sub>, and transport is measured by K<sub>1FLT</sub>:

$$\text{Flux constant: } K_{FLT} = K_{1FLT} \times k_{3FLT} / (k_{2FLT} + k_{3FLT}).$$

Preliminary modeling work from investigators in Seattle is illustrated in the model diagrams above (Muzi et al., 2005a, Muzi et al., 2005b). The kinetic modeling requires dynamic imaging and arterial plasma sampling or sampling via the heated hand method for arterialization of venous blood (Phelps et al., 1979).

To date there have been no human studies assessing the use of [<sup>18</sup>F]FLT to assess response to chemoradiation in primary high-grade brain tumors. Several studies have been performed assessing brain tumor treatment response in patients receiving chemotherapy and/or bevacizumab (Chen et al., 2007, Schwarzenberg et al., 2012, Wardak et al., 2014). A single study by Chen (Chen et al., 2007) used [<sup>18</sup>F]FLT to predict response in malignant glioma in patients receiving Bevacizumab and Irinotecan as second line therapy. The patients were treated with biweekly cycles of bevacizumab and irinotecan and were prospectively studied with [<sup>18</sup>F]FLT-PET at baseline, after 1 to 2 weeks, and after 6 weeks from start of treatment. A more than 25% reduction in tumor [<sup>18</sup>F]FLT uptake as measured by standardized uptake value was defined as a metabolic response. In this study there were nine responders (47%) and 10 non-responders (53%). Responders survived three times as long as non-responders (10.8 v 3.4 months;  $P = .003$ ), and tended to have a prolonged progression-free survival ( $P = .061$ ). Both early and later [<sup>18</sup>F]FLT -PET responses were more significant predictors of overall survival (1 to 2 weeks,  $P = .006$ ; 6 weeks,  $P = .002$ ), compared with the MRI responses ( $P = .060$  for both 6-week and best responses). A second study was published as an extension of the Chen study assessing response using [<sup>18</sup>F]FLT and [<sup>18</sup>F]FDOPA. This study showed the superiority of using kinetic modelling and multiple linear regression analysis to improve the predictive ability of the radiopharmaceuticals. Kinetic parameters from FLT were more predictive of overall survival than those from FDOPA. Using information from both radiopharmaceuticals resulted in a better three predictor model (adjusted R<sup>2</sup> = 0.83) than using information from FDOPA alone (adjusted R<sup>2</sup> = 0.41), and only marginally different from using information from FLT alone (adjusted R<sup>2</sup> = 0.82). Another study by Schwarzenberg (Schwarzenberg et al, 2012) assessed early survival prediction in patients receiving bevacizumab chemotherapy. In this study early and late changes in tumor [<sup>18</sup>F]F-FLT uptake were more predictive of overall survival than MRI criteria ( $P < 0.001$  and  $P = 0.01$ , respectively). [<sup>18</sup>F]F-FLT uptake changes were also predictive of progression-free survival ( $P < 0.001$ ). The median overall survival for responders was 3.3 times longer than for non-responders based on [<sup>18</sup>F]F-FLT PET criteria (12.5 vs. 3.8 months,  $P < 0.001$ ). A potential limitation of these studies was the use of standardized uptake value rather than full kinetic analysis in brain tumors (Peck et al., 2015). This is due to the fact that [<sup>18</sup>F]FLT is restricted at the blood-brain-barrier. This results in a transport-dominated SUV image because transport of [<sup>18</sup>F]FLT is the rate-limiting path to uptake. Studies utilizing full kinetic analysis would be helpful to test whether there

is any uptake of [<sup>18</sup>F]FLT by passive diffusion, allowing phosphorylation to be the rate determining step in the metabolism of [<sup>18</sup>F]FLT (Peck et al., 2015).

#### 2.4.2. [<sup>18</sup>F]Fluciclovine as an Imaging Agent

Anti-FACBC is a synthetic L-leucine analogue that is transported into cells but is not incorporated into proteins. It can be labelled with <sup>18</sup>F for PET imaging of amino acid transport and is thus known as [<sup>18</sup>F]Fluciclovine. It exhibits excellent *in vitro* uptake into the DU145 prostate carcinoma cell line and into orthotopic implanted prostate tumors in nude mice (Oka et al., 2007). The uptake of [<sup>18</sup>F]Fluciclovine is mediated via the amino acid transporter (AAT) responsible system. [<sup>18</sup>F]Fluciclovine uptake has been investigated in cell culture (Oka et al., 2012, Oka et al., 2014) and gene knock down studies (Okudaira et al., 2011). The sodium-dependent transporter ASCT2 and the sodium-independent system LAT1 have been identified as the dominant transporters involved (Ono et al., 2013). These findings are further corroborated using heterologous expression systems (Okudaira et al., 2013). Since amino acid uptake is enhanced in malignancy, it accumulates faster and to a higher degree in cancer cells than normal cells, making it a promising biomarker for cancer diagnosis by PET imaging. Only a small fraction is excreted through the urinary pathway in the first hours after injection and it is not taken up to a high degree by normal brain tissue; therefore, unlike [<sup>18</sup>F] fluorodeoxyglucose (FDG), PET imaging with [<sup>18</sup>F]Fluciclovine may be useful for the detection and diagnosis of prostate cancer and malignant gliomas. The ability of [<sup>18</sup>F]Fluciclovine to detect malignant lesions in humans has been assessed in preliminary studies by the Emory group and others. [<sup>18</sup>F]Fluciclovine is reported to have utility in the evaluation of primary and metastatic cancers in the brain, due to its low brain background uptake and high uptake by brain tumors (Shoup et al., 1999, Akhurst et al., 2006, Meirelles et al., 2006). More recently, it has been reported that PET imaging with [<sup>18</sup>F]Fluciclovine is useful in assessing metastatic or recurrent prostate cancer in the prostate bed, lymph nodes and bone (Schuster et al., 2007, Nanni et al., 2013, Ono et al., 2015a). The data suggest that the LAT system is highly active in these types of malignancies and that [<sup>18</sup>F]Fluciclovine is a promising radiotracer for PET imaging of brain and prostate tumors.

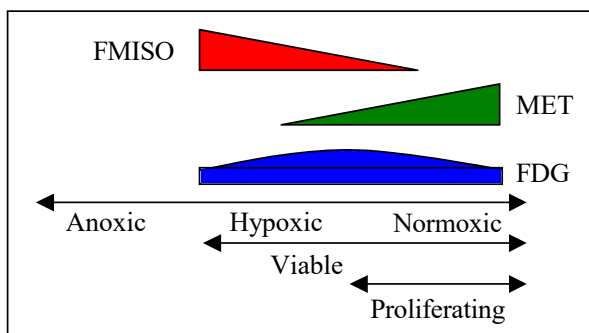
To date there have been no published human studies assessing the use of [<sup>18</sup>F]Fluciclovine to assess response to chemoradiation in glioma. In an autoradiography animal study by Ono (Ono et al., 2015b) utilizing anti-<sup>14</sup>C-FACBC (rather than [<sup>18</sup>F]Fluciclovine), there was a significant reduction in anti-<sup>14</sup>C-FACBC uptake (represented by the differential absorption ratio (DAR)) in animals treated with chemotherapy. In these experiments a human xenograft rat glioma model utilizing U87 and U87R (TMZ-resistant subculture) cells were inoculated into the right and left basal ganglia. Single agent and combination therapy was performed with temozolomide, interferon- $\beta$ , and bevacizumab. Tumor to non-tumor ratios (T/NT) were also determined. The DAR of anti-<sup>14</sup>C-FACBC was significantly higher in tumors than in non-involved cortices. <sup>14</sup>C-FACBC accumulation was lower after single-agent treatment and lower still after combination therapy. Treatment with temozolomide alone significantly decreased the T/NT for the DAR of U87 tumors, whereas the T/NT ratios for those of U87R tumors remained unchanged. Temozolomide and interferon- $\beta$  combination treatment significantly decreased anti-<sup>14</sup>C-FACBC uptake in both U87R and U87 tumors. Addition of bevacizumab to the temozolomide and interferon- $\beta$  combination further decreased anti-<sup>14</sup>C-FACBC uptake in both tumor types. In another study, Doi (Doi et al., 2015) showed that [<sup>18</sup>F]Fluciclovine-PET visualization of gliomas was possible and occurred irrespective of blood-brain integrity. This is important since radiopharmaceuticals that cross the BBB, such as Fluciclovine, will be taken up by low-grade tumors which in many instances will have equivocal findings with MRI due to an intact blood-brain barrier. In the study, the tumor-to normal brain uptake ratio of [<sup>18</sup>F]Fluciclovine for the most part correlated with tumor cell density. The authors felt that [<sup>18</sup>F]Fluciclovine PET combined with MRI will be valuable for preoperative glioma delineation. The authors reiterated the fact that surgery will continue to be the first-line therapy for both low-grade and high-grade gliomas. With its unique ability to show uptake

even with an intact blood-brain barrier, [<sup>18</sup>F]Fluciclovine has the ability to delineate the extent of tumor in low-grade tumors. Improved delineation of the tumor, particularly low-grade tumors, will have potential clinical utility.

## 2.5. Complementary Value of Multiple PET Tracers

There is a significant and growing body of evidence that complementary imaging of more than one tracer (“multi-tracer PET”) would provide enhanced information over FDG imaging alone for many cancer imaging applications (Wong et al., 2002). We highlight certain examples:

- Kubota *et al.* (Kubota et al., 1993) studied <sup>18</sup>F-FDG or <sup>18</sup>F-fluorouridine, <sup>14</sup>C-methionine, <sup>3</sup>H-thymidine, and <sup>67</sup>Ga-citrate in rat tumors, finding differing and complementary information for each tracer. Similar work by d’Argy *et al.* (d’Argy et al., 1988) with FDG, thymidine, methionine, and toremifene (an estrogen receptor-avid agent), also demonstrated complementary value of these tracers.
- Mankoff *et al.* (Mankoff et al., 2002, Mankoff et al., 2003) found that the *ratio* of the metabolic rate of FDG to blood flow (from <sup>15</sup>O-water) was the best predictor of macroscopic complete response of advanced breast cancer to neoadjuvant chemotherapy, and that this *ratio* is a more robust indicator of tumor status than either tracer alone.
- Kubota *et al.* (Kubota et al., 1999) evaluated the imaging potential of agents for hypoxia (<sup>18</sup>F-MISO), glucose metabolism (<sup>14</sup>C-2-deoxyglucose) and proliferation (<sup>11</sup>C-Met) in a rat tumor model:  
*“Combined use of these tracers might provide better information on tumor characteristics. The ratio of FMISO uptake to 2DG (FDG) uptake may reflect the proportion of hypoxic tissue in the tumor, the hypoxic fraction. This fraction has been used in the field of radiation biology as an important index to predict radiosensitivity. On the other hand, the ratio of Met uptake to 2DG (FDG) uptake may correspond to the percentage of proliferative tissue, the growth fraction. This is also an important parameter with which to describe the growth kinetics of tumor tissue.”*



**Figure 1.** Distribution model proposed by Kubota *et al.* (Kubota et al., 1999) highlighting the complementary value of multi-tracer PET imaging using tracers for hypoxia, proliferation, and glucose metabolism. The combinations of these three tracers provides the hypoxic fraction and growth fractions of the tumor, which will enhance grading prognosis, and especially treatment planning and monitoring with PET.

- Dimitrakopoulou-Strauss *et al.* (Dimitrakopoulou-Strauss et al., 2001) studied imaging with FDOPA, FDG, and <sup>15</sup>O-water in melanoma patients, finding that detectability of metastases was enhanced when both FDOPA and FDG were used. The accompanying editorial (Graham et al., 2003) highlights the potential of multi-tracer PET.
- In non-Hodgkin’s lymphoma, Leskinen-Kallio *et al.* (Leskinen-Kallio et al., 1991) found that <sup>11</sup>C-methionine (MET) was preferable for detection, but FDG was superior for distinguishing tumor grade. Aronen *et al.* (Aronen et al., 2000) likewise found that MET gave better detection and FDG better grading in brain tumors.

- Narayanan *et al.* (Narayanan et al., 2002) found that combined FDG + MET was useful for grading astrocytomas, and MET was better than FDG for delineating low-grade tumor boundaries. The same effect was observed by Heiss *et al.* (Heiss et al., 1996), with amino acid tracers (<sup>11</sup>C-MET, <sup>18</sup>F-tyrosine) offering better detection and FDG better grading.
- In prostate cancer, Oyama *et al.* (Oyama et al., 2002) found <sup>11</sup>C-acetate (ACE) more sensitive for primary disease and local metastases, and FDG preferable for distant metastases and tumor grade.
- In hepatocellular tumors, Ho *et al.* (Ho et al., 2003) found that ACE had a sensitivity of 87.3%, compared to only 47.3% for FDG. Well differentiated lesions had high lesion-to-liver uptake ratios by ACE and low by FDG, whereas poorly differentiated lesions had high FDG uptake and low ACE uptake.
- PET can detect physiologic changes in tumors that occur immediately in response to therapy, providing early (subclinical) assessment of the effectiveness of therapy (reviewed in (Young et al., 1999, Hoekstra et al., 2000, Lammertsma, 2001, Stokkel et al., 2001, Van der Hiel et al., 2001, Eary et al., 2002)). Much of this work has focused on FDG-PET (Ichiya et al., 1991, Chaiken et al., 1993, Blasberg, 1994, Letocha et al., 1994, Price et al., 1995, Findlay et al., 1996, Ichiya et al., 1996, Jones et al., 1996, Mortimer et al., 1996, Mankoff et al., 1997, Mitsuhashi et al., 1997, Schulte et al., 1999, Franzius et al., 2000, Nair et al., 2000, Vlassenko et al., 2000, Krohn et al., 2001, Mortimer et al., 2001, Mankoff et al., 2002, Mankoff et al., 2003), but Eary *et al.* (Eary et al., 2002) and Lammertsma (Lammertsma, 2001) highlight the importance of imaging cellular proliferation, perfusion and hypoxia in addition to glucose metabolism for this task.

### **3. PHARMACOLOGY AND SAFETY OF [<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine and RADIATION DOSIMETRY OF [<sup>18</sup>F]Fluciclovine and [<sup>18</sup>F]FLT**

#### **3.1. Pharmacology and Safety**

##### 3.1.1. Pharmacology of [<sup>18</sup>F]FLT in Human

The pharmacology of FLT is based on its action as an inhibitor of DNA synthesis (Langen et al., 1968, Langen et al., 1971, Langen and Graetz, 1972, Matthes et al., 1987, Matthes et al., 1988). Intracellular metabolism of FLT produces FLT phosphates, but these nucleotides inhibit endogenous DNA polymerases because they lack a 3'-hydroxyl substituent. This results in premature chain termination for DNA (Matthes et al., 1987, Sundseth et al., 1996). These biochemical properties can account for FLT's prominent hematological and liver toxicity (Faraj et al., 1994, Flexner et al., 1994, Sundseth et al., 1996). The pharmacology of FLT is similar to that of the widely used prescription HIV-antiviral drug azidothymidine (AZT) (Lundgren et al., 1991, Kong et al., 1992). Both FLT and AZT are 3'-deoxythymidine analogs that act as inhibitors of DNA synthesis and are cleared from the body in the same way. However, FLT was significantly more cytotoxic than AZT in test cell lines (Faraj et al., 1994). Cellular uptake of FLT and thymidine was greater than that of AZT. Transport of FLT and thymidine across cell membranes occurs both by active transport and by simple diffusion (Kong et al., 1992).

##### 3.1.2. Toxicity of FLT in Humans

FLT was investigated as an anti-AIDS drug in humans (Flexner et al., 1994). Toxic effects and death were reported for some subjects receiving FLT during randomized concentration controlled trials during a 16-week treatment of oral multi-dosing. Doses of 0.125 mg/Kg every 12h, produced a mean cumulated drug exposure (AUC<sub>12</sub>: area under curve) of 417 ng-h/mL. At this level, serious (grade 3) hematologic toxicity

occurred in 6 of 10 subjects. At 300 ng-h/mL, grade 2 or greater (fall in hemoglobin to < 9.4 g/dL) developed within 4 weeks in 9 of 12 subjects. At 200 ng-h/mL almost no clinically significant anemia developed, but dose-limiting granulocytopenia (< 750 granulocytes/mm<sup>3</sup>) occurred in 5 of 15 subjects. Mild peripheral neuropathy occurred in 2 of 10 subjects at 50 ng-h/mL, but was not dose-limiting. FLT drug trials were terminated after the unexpected death of 2 subjects of hepatic failure. One patient assigned to 200 ng-h/mL developed progressive liver failure and died after 12 weeks of FLT therapy. A second subject, who received a fixed dose of 10 mg/day, developed progressive liver failure and died at about the same time. All surviving subjects were followed closely for 4 weeks after stopping FLT and none had evidence of clinically significant liver disease or other adverse effects. Overall, 25 of the 44 subjects receiving at least two doses of FLT, completed the 16 week study without clinically significant adverse effects.

In the human studies of [<sup>18</sup>F]FLT, no toxicities were reported at the doses and specific activities provided in numerous publications. However, it must be emphasized that of the 26 papers reviewed as part of the NCI initial IND submission for [<sup>18</sup>F]FLT only half (n=13) mentioned specific activity of the [<sup>18</sup>F]FLT. None of these published manuscripts specifically mentioned whether any assessments of safety or toxicity were performed.

In a recent study performed at the University of Washington, Turcotte and colleagues (Turcotte et al., 2007) preliminarily assessed the toxicity of [<sup>18</sup>F]FLT in twenty patients with proven or suspected diagnosis of non-small cell lung cancer. All patients gave written informed consent to the [<sup>18</sup>F]FLT injection, subsequent PET imaging, and blood draws. Blood samples were collected for each patient at multiple times before and after [<sup>18</sup>F]FLT-PET. These samples were assayed for comprehensive metabolic panel, total bilirubin, and complete blood and platelet counts. In addition, a standard neurological examination was performed for each patient by a qualified physician before and immediately after [<sup>18</sup>F]FLT-PET. All [<sup>18</sup>F]FLT doses were calculated based on patient weight (2.59MBq/kg = 0.07 mCi/kg) with a maximal dose of 185 MBq (5mCi). Starting with the [<sup>18</sup>F]FLT injection, dynamic PET images were acquired for 90 or 120 minutes. By placing a region-of-interest in the center of the left ventricular chamber, blood time-activity curves were generated for each patient from the dynamic PET data and then extrapolated to 720 minutes. This provided a measure of the area under the [<sup>18</sup>F]FLT concentration curve for 12 hours (AUC<sub>12</sub>). A separate estimation of the AUC<sub>12</sub> was also obtained from sequential blood samples collected during PET data acquisition. The AUC<sub>12</sub> values estimated from imaging data were not significantly different from those found from serial measures of [<sup>18</sup>F]FLT blood concentrations (p= 0.66). No side effects were reported by patients or witnessed. No change was observed in the neurological status of patients. Only albumin, red blood cell count, hemoglobin, and hematocrit showed a statistically significant decrease over time. These changes were attributed to IV hydration during PET imaging and to subsequent blood loss at surgery.

The single dose AUC<sub>12</sub> values derived from blood samples ranged from 0.22 to 1.34 ng-h/mL with a mean of 0.80 ng-h/mL. This range corresponds to 0.46% to 2.7% of the Flexner clinical trial AUC<sub>12</sub> of 50 ng-h/mL. The AUC<sub>12</sub> of a single 10 mCi radiotracer dose of [<sup>18</sup>F]FLT is small compared to the AUC<sub>12</sub> of the least toxic therapeutic trial dose. This is because the Flexner trial administered [<sup>18</sup>F]FLT every 12 hours for 112 days with the only toxicity, peripheral neuropathy, developing at a mean of 40 days. At the lowest acceptable [<sup>18</sup>F]FLT specific activity (200 Ci/mmol), the [<sup>18</sup>F]FLT dose from 10 mCi is ≤ 24.4 μg (< 50 nmol) for one [<sup>18</sup>F]FLT PET study. The maximum dose from three 10.0 mCi injections of [<sup>18</sup>F]FLT in a 70 kg subject, the maximum number doses that a single subject could receive under this protocol, is <20% of the 12 hour dose and less than 0.2% of the cumulative 40 day dose associated with any reported toxic effect in humans. The only reported toxic effect was a mild, non-dose limiting peripheral neuropathy in 2/10 subjects at 50 ng-h/mL from oral administration of FLT every 12 hour for 16 weeks. Based on these data, we conclude that FLT imaging will not lead to clinically detectable toxic effects. In addition, a recent small study assessing possible toxicities of [<sup>18</sup>F]FLT in primary brain tumor patients was also published from

investigators at the University of Washington (Spence et al., 2008). No significant toxicities to [<sup>18</sup>F]FLT were noted in this twelve patient study.

There is some literature on the mutagenic properties of FLT. Ehrlich ascites tumor cells incubated with 10  $\mu$ M FLT for extended periods (12, 24, 36 h: AUC 120, 240, 360 nmol-h/mL) showed chromosome damage (Bäumlein and Wobus, 1976). The most prominent effects were breaks and gaps, however, much less damage was seen if a recovery time was included (12, 24 h) and the damage could also be largely reversed by post-treatment with thymidine (10  $\mu$ M). FLT anabolism, FLT incorporation into DNA, and the effects of FLT on cellular genome integrity have been studied in cultured CEM (CD4<sup>+</sup> human lymphoblastoid) cells. FLT concentrations of 10 and 100  $\mu$ M produced chromosome fragmentation characteristic of cells undergoing apoptosis. In contrast, at 1  $\mu$ M FLT, the level of fragmentation was similar to the controls without FLT exposure. Despite prominent levels of intracellular FLT anabolites, the fraction of FLT in DNA was low ( $10^{-6}$  total). At the lowest acceptable [<sup>18</sup>F]FLT specific activity (200 Ci/mmol), the dose to patients ( $\leq 10$  mCi) will correspond to an initial, maximal plasma concentration of about 10 nM. This is 100 times lower than the level of FLT where no chromosomal damage was seen in CEM cells (1  $\mu$ M). Based on these data, the administration of 10 mCi of [<sup>18</sup>F]FLT to humans does not pose a probable threat of mutagenesis.

### 3.1.3. Pharmacology of [<sup>18</sup>F]Fluciclovine in Humans

The information provided here in is abstracted from the [<sup>18</sup>F]Fluciclovine Investigator Drug Brochure Version 5 (2017) and Axumin prescribing information (Axumin 2016). Three studies have evaluated biodistribution and radiation dosimetry in healthy volunteers (Nye et al., 2007, Asano et al., 2011, McParland et al., 2013). In general, the results of these studies are consistent. The primary evidence on the biodistribution and radiation dosimetry from the phase I study conducted by GE Healthcare (McParland et al., 2013, Sørensen et al., 2013) is reviewed in more detail below. This trial (GE-148-001) in 6 healthy volunteers and 6 subjects with biopsy-proven prostate cancer started in June 2009 and the clinical phase was completed in November 2009. The first part of the trial investigated the safety, biodistribution, and internal radiation dosimetry of [<sup>18</sup>F]Fluciclovine injection in 6 healthy volunteers (3 males and 3 females). Overall, there was no evidence indicating that [<sup>18</sup>F]Fluciclovine is metabolized *in vivo*.

Qualitative assessment of the distribution of [<sup>18</sup>F]Fluciclovine activity in all 6 subjects showed that the distribution was largely uniform throughout the body with the exception of the liver, pancreas, lung, red bone marrow, and heart wall. The initial uptake of [<sup>18</sup>F]Fluciclovine in each subject was assessed at the first imaging time point, on average 7.9 mins (6.5 to 10.2 mins) post injection. Initial uptake of [<sup>18</sup>F]Fluciclovine activity in the liver, pancreas, and the red bone marrow of the thoracic and lumbar vertebrae and skull was immediately evident in all subjects. There was very little brain uptake, 1.6% (0.7% to 2.2%). With increasing time post injection, distributed uptake was apparent and, on the basis of the anatomical distribution, was mostly associated with skeletal muscle. This uptake could not be isolated for quantification as it was not possible to segment the muscle in the whole-body image. Hence, muscle was included in the “remaining tissues” category. Uptake in the spleen was apparent in some subjects. Uptake in the uterus was apparent in some female subjects and initial activity uptake in the uterus was limited, 1.2% (0.3% to 1.7%). It was assumed, based on the general skeletal muscle uptake, to be associated with the muscle of the uterine wall. The four organs with the highest initial uptake of [<sup>18</sup>F]Fluciclovine were the liver at 13.8% (8.3% to 17.1%), red bone marrow at 11.1% (4.8% to 20.4%), lung at 7.1% (5.2% to 8.6%), and pancreas at 4.2% (3.4% to 5.2%). Based on comparison with washout of activity from the whole-blood samples, washout of activity from the lung was consistent with washout of activity from the pulmonary blood content.

Excretion of [<sup>18</sup>F]Fluciclovine activity was limited and was only found for the renal pathway, reaching 3.2% (1.1% to 7.4%) at the last imaging time point an average of 4.2 hours post injection (3.8 to 4.7 hours). The small amount of activity entering the gastrointestinal (GI) tract could not be clearly identified due to the surrounding activity in muscle and mesentery. On the basis of activity washout from the liver and the pancreas, and assuming that all of this activity was to enter the duodenum through hepatobiliary and pancreatic transport, the activity excreted via the GI tract was estimated to be of the order of 10 % or less. Although this could not be accurately quantified, any activity present in the contents of the GI tract was grouped within the “remaining tissues” category for evaluation of internal radiation dosimetry. No differences considered to be of likely clinical significance were noted between the 3 males and the 3 females.

#### 3.1.4. Toxicity of [<sup>18</sup>F]Fluciclovine in Humans

The information provided here in is abstracted from the [<sup>18</sup>F]Fluciclovine Investigator Drug Brochure, Version 5 (2017) and Axumin prescribing information (Axumin 2016). Fourteen-day repeat-dose toxicity studies in two mammalian species (rat and dog) showed no important treatment-related adverse effects. Genotoxicity tests showed that no mutagenic or clastogenic effects were elicited by [<sup>18</sup>F]Fluciclovine or its major impurity, AH114760. No adverse effects were observed during local tolerance tests using the intravenous and paravenous administration route in rabbits. [<sup>18</sup>F]Fluciclovine also showed no potential for hemolysis of human blood.

**Pregnant or nursing women:** Reproductive toxicology studies have not been performed with [<sup>18</sup>F]Fluciclovine. However, since [<sup>18</sup>F]Fluciclovine is a radiopharmaceutical, it is assumed that fetal toxicity or embryo fetal toxicity may result if it is administered to a pregnant woman. Therefore, [<sup>18</sup>F]Fluciclovine injection is not suitable for administration to pregnant or nursing women.

**Genotoxic and mutagenic potential:** The results of mutagenicity and clastogenicity studies performed with [<sup>18</sup>F]Fluciclovine, including the Ames, MLA and rat *in vivo* bone marrow micronucleus assays, did not reveal either mutagenic or clastogenic potential. No carcinogenicity studies have been conducted, because [<sup>18</sup>F]Fluciclovine injection is intended as a diagnostic imaging agent for infrequent administration.

**Findings in safety pharmacology and toxicology studies:** Nonclinical toxicology and safety pharmacology studies with [<sup>18</sup>F]Fluciclovine have not identified any specific target organs or adverse effects on nervous, respiratory, or cardiovascular systems. As such, no potential [<sup>18</sup>F]Fluciclovine injection-related safety risks for humans have been identified on the basis of the nonclinical data.

**Expected risks to subjects participating in clinical studies:** No serious adverse reactions have been reported from any investigator sponsored studies or from the compassionate use program in Norway. The risks to subjects mainly relate to the intravenous injection and intravenous blood sampling procedures, and the radiation emitted by [<sup>18</sup>F]Fluciclovine. Intravenous injection and the use of an intravenous cannula are known to carry a small risk of infection and hematoma. The exposure to radiation will not exceed that which is considered acceptable in accordance with appropriate guidelines.

AEs arising from the industry-sponsored studies described above which are considered to be potential adverse reactions to [<sup>18</sup>F]Fluciclovine are listed in Table 1. Of these events, dysgeusia, headache, redness at the injection site, and sensation of burning during injection are considered to be adverse drug reactions potentially related to the investigational product and are included in the summary Table 1 below (Reference Safety Information). [<sup>18</sup>F]Fluciclovine remains under clinical investigation. Investigators should be alert to the potential for additional adverse reactions to be observed as clinical experience continues



(<sup>18</sup>F)Fluciclovine Investigator’s Brochure, Version 05; May 30, 2017 and Axumin prescribing information 2016). Based on the pharmacokinetics of [<sup>18</sup>F]Fluciclovine and of AH114760, the safety reporting window for monitoring for potential adverse reactions is set at 28 days post injection.

Table 1. Reference Safety Information

System organ class/Preferred term	Frequency Number of Subjects (%) Total N = 1153	Intensity		
		Mild	Moderate	Severe
<b>Any Event</b>	<b>12 (1%)</b>	<b>12</b>	<b>0</b>	<b>0</b>
<b>General disorders and administration site conditions</b>	<b>7(0.6%)</b>	<b>7</b>	<b>0</b>	<b>0</b>
Injection-site reactions	7 (0.6%)	7	0	0
<b>Nervous system disorders</b>	<b>4 (0.3%)</b>	<b>4</b>	<b>0</b>	<b>0</b>
Dysgeusia	4 (0.3%)	4	0	0
<b>Respiratory Organ, Thoracic and Mediastinal Disorders</b>	<b>2 (0.2%)</b>	<b>2</b>	<b>0</b>	<b>0</b>
Parosmia	2 (0.2%)	2	0	0

\*1 patient reported both dysgeusia and parosmia

### 3.2. Human Radiation Dosimetry

The information on dosimetry calculations for each individual tracer is presented in sections 3.2.1 and 3.2.2. The CT dosimetry calculations used for attenuation correction used in conjunction with the PET imaging is provided in section 3.2.3. The cumulative dosimetry (Table 2) for a maximum of 6 possible PET/CT examinations (baseline imaging of each tracer, imaging of each tracer after completion of therapy, and imaging of each tracer at the time of pseudoprogression) is then presented in section 3.2.4. The dosimetry for both [<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine is based on published studies and the biodistribution and radiation dosimetry provided in the [<sup>18</sup>F]Fluciclovine Investigator Drug Brochure, Version 5, 5/30/2017 and Axumin prescribing information 2016. A CT is required for attenuation correction and the dosimetry tables include the available data that has been revised based on ICRP 106.

#### 3.2.1. Human Radiation Dosimetry of [<sup>18</sup>F]FLT

Eighteen patients (11 men, 7 women) with known or suspected lung cancer were prospectively studied with [<sup>18</sup>F]FLT-PET imaging at the University of Washington from March 2000 to April 2002 (Vesselle et al., 2003). Biodistribution data from these 18 patient studies were used for dosimetry calculations. The amount of injected [<sup>18</sup>F]Fluorothymidine activity used in this protocol will be approximately 370 MBq (10 mCi). The dosimetry for this compound was previously published in ICRP Publication 128 (Mattsson, 2015). The greatest organ absorbed doses for a 370 MBq (10 mCi) injection of [<sup>18</sup>F]Fluorothymidine are the liver (18 mGy) and the kidneys (16 mGy), while the effective dose is 5.6 mSv (Table 2).

### 3.2.2. Human Radiation Dosimetry of [<sup>18</sup>F]Fluciclovine

Three studies have evaluated biodistribution and radiation dosimetry in healthy volunteers (Nye et al., 2007, Asano et al., 2011, McParland et al., 2013). In general, the results of these studies are consistent. The primary evidence on the biodistribution and radiation dosimetry from the phase I study conducted by GE Healthcare (McParland et al., 2013, Sørensen et al., 2013) was used to determine the human radiation dosimetry. The amount of injected [<sup>18</sup>F]Fluciclovine activity used in this protocol will be approximately 370 MBq (10. mCi). A summary of dosimetry studies for this compound was previously published and is used to estimate radiation dose and risks in this protocol (McParland et al., 2013). The Blue Earth Diagnostics Investigator's Drug Brochure for [<sup>18</sup>F]Fluciclovine also uses the dosimetry from this paper. The greatest organ absorbed doses for a 370 MBq (10 mCi) injection of [<sup>18</sup>F]Fluciclovine are the pancreas (38 mGy) and the heart (19 mGy), while the effective dose is 8.2 mSv (Table 2).

### 3.2.3 Human Radiation Dosimetry for CT-based Attenuation Correction for PET

The study will be performed on the research GE Discovery 710 PET/CT scanner in the Molecular Imaging Suite at HCI. Each PET imaging study will require CT imaging for attenuation correction. After positioning the patient in the gantry of the PET/CT system, a topogram of the head will be obtained to confirm the correct positioning and identify the anatomic range for a single PET bed position covering the entire brain. A helical CT scan is then performed over the same anatomic range corresponding to the PET scan in order to perform for attenuation correction. The PET emission scan will commence immediately after the CT scan. The CT acquisition parameters will be 120 kVp, 0.5s rotation speed, 35 mA tube current, 64 x 0.625 mm collimation, and a pitch of 0.984. This results in a CT Dose Index (CTDI<sub>vol</sub>) of 2.81 mGy based on a 16 cm phantom. A standard length of 1 PET bed position results in a Dose Length Product (DLP) of 55.3 mGy x cm. The estimated effective dose using the DLP scaling method is 0.12 mSv (AAPM Report No. 96, 2008). In order to estimate the absorbed doses of individual organs and the resulting effective dose, the ImpACT Scan CT Patient Dosimetry Calculator (Version 1.0.4) was used with the specific acquisition parameters used in this protocol and the NRPB Monte Carlo dose data sets for the GE Lightspeed VCT scanner produced in report SR250 dosimetry tables (Jones et al., 1991). A correction factor has been applied to account for the difference in the CTDI<sub>vol</sub> derived from ImpACT Calculator and that reported on the GE scanner. Note that the topogram contributes a negligible radiation exposure to the helical CT exam and was not included in the dose estimates. The greatest organ absorbed doses for a single head CT scan using the aforementioned acquisition parameters are the eye lenses (2.4 mGy) and the brain (2.4 mGy), while the effective dose is 0.10 mSv (Table 2).

### 3.2.4. Cumulative Radiation Dosimetry for [<sup>18</sup>F]FLT/CT + [<sup>18</sup>F]Fluciclovine/CT

The dosimetry (mGy/10.0 mCi) for each radiotracer is summarized in Table 2. The proximate maximum administered activity for each tracer will be as follows: 10.0 mCi [<sup>18</sup>F]FLT and 10 mCi [<sup>18</sup>F]Fluciclovine. There is a maximum total of 6 PET/CT scans possible on this study. This includes baseline [<sup>18</sup>F]FLT/CT and [<sup>18</sup>F]Fluciclovine/CT, immediate post therapy [<sup>18</sup>F]FLT/CT and [<sup>18</sup>F]Fluciclovine/CT, and [<sup>18</sup>F]FLT/CT and [<sup>18</sup>F]Fluciclovine/CT at the time of pseudo-progression. A group of patients may only receive the [<sup>18</sup>F]FLT/CT and [<sup>18</sup>F]Fluciclovine/CT at the time of pseudo-progression. A low-dose CT is used for attenuation correction and will be obtained for each PET study.

Table 2. Cumulative Radiation Dosimetry

<b>IRB #104740</b>	<b>[<sup>18</sup>F]Fluciclovine -PET</b>	<b>[<sup>18</sup>F]FLT-PET</b>	<b>Low Dose Head CT</b>	<b>PET/CT Total</b>
<b>Activity (mCi)</b>	10	10	-	-
<b>Number of Scans:</b>	3	3	6	
<b>Organ</b>	<b>Single Scan Absorbed dose (mGy)</b>	<b>Single Scan Absorbed dose(mGy)</b>	<b>Single Scan Absorbed dose(mGy)</b>	<b>Protocol Absorbed dose (mGy)</b>
<b>Adrenals</b>	5.99	5.92	0.000367	35.74
<b>Bladder</b>	9.32	8.51	0.000009	53.50
<b>Bone surfaces</b>	8.58	7.03	0.626846	50.60
<b>Brain</b>	3.22	3.03	2.377692	33.03
<b>Breasts</b>	5.07	3.03	0.001513	24.32
<b>Gallbladder</b>	6.18	6.66	0.000069	38.52
<b>Stomach</b>	5.18	4.44	0.000223	28.86
<b>Small intestine</b>	4.88	4.81	0.000013	29.08
<b>Colon</b>	4.70	4.44	0.000011	27.42
<b>Heart</b>	19.13	4.44	0.001441	70.72
<b>Kidneys</b>	5.07	15.91	0.000159	62.94
<b>Liver</b>	12.40	17.76	0.000360	90.47
<b>Lungs</b>	12.77	4.07	0.005260	50.54
<b>Muscles</b>	3.92	3.63	0.051156	22.95
<b>Oesophagus</b>	4.63	3.63	0.003603	24.77
<b>Ovaries</b>	4.92	4.44	0.000000	28.08
<b>Pancreas</b>	37.81	5.55	0.000245	130.09
<b>Red marrow</b>	9.14	9.62	0.151308	57.18
<b>Skin</b>	2.96	2.78	0.158513	18.16
<b>Spleen</b>	8.81	8.14	0.000346	50.84
<b>Testes</b>	6.44	3.26	0.000000	29.08
<b>Thymus</b>	4.63	3.63	0.003603	24.77
<b>Thyroid</b>	3.81	3.48	0.100872	22.47
<b>Uterus</b>	16.50	4.44	0.000009	62.83
<b>Effective dose (mSv)</b>	8.18	5.55	0.10	41.79

## 4. RATIONALE, GOALS AND OBJECTIVES

### 4.1. Clinical Rationale

The standard treatment approach for patients with high-grade primary brain tumors includes maximum feasible surgical resection, followed by 6 weeks of concurrent cranial irradiation, and daily low-dose temozolomide chemotherapy; followed by 12 cycles of high-dose temozolomide administered for 5 consecutive days every 4 weeks (Stupp et al., 2005). Contrast-enhanced MRI is the current standard for evaluating the success of therapy and monitoring for tumor recurrence. MRI is typically obtained prior to initial surgery, within 24 hours after surgery, at the conclusions of cranial irradiation, and then every 8 weeks during temozolomide chemotherapy until evidence of recurrence. Despite this careful clinical and radiographic surveillance, and despite decades of research into the histologic and molecular classification of primary brain tumors, our ability to predict tumor behavior remains very limited. Some gliomas will result in overall survival times of only months, whereas other histologically-identical gliomas may yield survivals of years to decades (Curran et al., 1993, Carson et al., 2007). Current assessment of tumor response to therapy is also poor. Patients with complete radiographic response after cranial irradiation often progress rapidly post-irradiation. In contrast, some patients with enhancing masses at the end of chemoradiotherapy may respond dramatically to further chemotherapy alone; or the masses may even disappear in the absence of further therapy, so called “tumor pseudoprogression” (Chamberlain et al., 2007). This confounding situation demonstrates a need for better assessment of tumor response.

Improvements in the ability to predict tumor behavior prior to the start of therapy would allow more efficient and effective tumor surveillance; better prognostication; and more appropriate assignment of patients to conventional, aggressive, or investigational therapies early in their clinical courses. This would provide huge economic and social benefits, and could afford decisive insights into brain tumor physiology and biology. Similarly, the ability to identify, earlier and more accurately, whether individual patients were responding to therapy would allow prompt discontinuation of ineffectual treatments and institution of potentially more effective therapies.

Previous efforts using imaging for such tasks have generally been limited to a single modality (e.g. MRI) and/or single-tracer (e.g. FDG-PET). However, there is a significant and growing body of evidence that complementary imaging of multiple aspects of tumor physiology (i.e. using multiple PET tracers) can provide greatly enhanced information over imaging with a single modality or tracer alone. In solid tumors, complex interactions exist between blood flow, metabolic activity, and oxygen status which affect metastatic and proliferative activity. Heterogeneous tumors may contain both slow-growing and fast-growing regions that present different profiles of proliferation rates and amino acid uptake.

### 4.2. Hypotheses

The hypothesis of this exploratory clinical trial in patients with high-grade a primary brain tumor who receive chemoradiation is that the PET imaging agents [<sup>18</sup>F]Fluciclovine and/or [<sup>18</sup>F]FLT will be a better predictor of tumor response than standard MRI based brain tumor response criteria. When used in conjunction, the two PET agents may be better able to predict tumor aggressiveness and thus overall survival than the use of individual-tracer PET biomarkers. This may eventually lead to improved assessment of response (including time to progression and overall survival) and differentiation of tumor recurrence/progression from treatment effect (pseudoprogression).

### 4.3. Exploratory Objectives

#### 4.3.1. Exploratory Objectives

The primary objective of the study is to show that a 25% reduction in SUV<sub>max</sub> or SUV<sub>mean</sub> for each of the PET imaging tracers will be predictive of overall survival

#### Secondary Exploratory Objectives

- Assess the association between overall survival and change in each of the following quantitative markers for [<sup>18</sup>F]Fluciclovine and [<sup>18</sup>F]FLT: K<sub>1-k4</sub>, K<sub>net</sub> and K<sup>trans</sup>, from baseline to post therapy.
- Assess the association between progression free survival and change in each of the following quantitative markers for [<sup>18</sup>F]Fluciclovine and [<sup>18</sup>F]FLT: K<sub>1-k4</sub>, K<sub>net</sub> and K<sup>trans</sup>, from baseline to post therapy.
- Assess the ability of [<sup>18</sup>F]FLT and/or [<sup>18</sup>F]Fluciclovine alone or together to predict true progression from pseudoprogression in patients with this clinical dilemma.
- A 25% reduction in SUV<sub>max</sub> or SUV<sub>mean</sub> for each of the PET imaging tracers will be more predictive of longer overall survival than the current MRI based response criteria.
- A 25% reduction in SUV<sub>max</sub> or SUV<sub>mean</sub> for each of the PET imaging tracers will be more predictive of time to progression than the current MRI based response criteria.

Two different groups of patients will be able to enroll in the trial. The first group, Group A, will be for patients who have not yet received any therapy for their suspected or newly found brain tumors. Group B will enroll patients with pathologically proven malignant brain tumor (WHO Grade III or IV glial-based tumors) who have undergone chemoradiation and have a contrast enhancing mass  $\geq 1.0$  cm in greatest diameter concerning for pseudoprogression. MRI-documented possible recurrence/progression versus treatment effect (pseudoprogression) within 6 months from the time of completion of chemoradiation.

Group A will have three different patient cohorts. The first, for patients where there is compelling evidence, based on the MRI and/or CT imaging, that a high-grade primary brain tumor is present. Pathologic confirmation will occur with subsequent biopsy or surgery. The second cohort of patients are individuals where the tumor is felt to be inoperable and a biopsy is performed but no surgery. The third cohort of patients are post-surgery where a complete surgical resection was not possible. This will be confirmed by a post-operative contrast MRI/CT scan where residual tumor  $\geq 1.0$  cm in diameter is present.

A number of quantitative and semi-quantitative imaging biomarkers for each tracer will be computed at each imaging time point, and the change in each biomarker between time-points will also be computed. These data will be compared with changes in tracer uptake before and after therapy and used to determine the semi-quantitative change in [<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine uptake which is consistent with response based on standard brain tumor response. In addition the imaging biomarkers will be used to determine if they are predictive endpoints (survival, time to progression), and with tumor biologic information (histology, WHO grade, vascularity, MIB-1, VEGF, EGFR, p53, IDH status) in cases when tumor tissue becomes available from standard care. These data will provide pilot information regarding the potential value of concurrent multiple PET biomarkers for predicting tumor behavior prior to the start of therapy, for improved prognostication, for more efficient and effective tumor surveillance, and/or for more appropriate assignment of patients to conventional, aggressive, or investigational therapies early in their clinical courses.

All patients will receive standard tumor-directed therapy and will be monitored with conventional imaging according to standard of care, which may include MRI and FDG-PET as described earlier. The [<sup>18</sup>F]FLT or

[<sup>18</sup>F]Fluciclovine PET research scans will *not* affect clinical decisions. A database of demographic, clinical, and molecular biologic information for each patient will be created. Tissue samples, when available from routine care, will undergo detailed histopathologic evaluations, with results prospectively and systematically recorded. All patients will be followed until the end of the trial (at least 12 months post last imaging assessment) for important clinical endpoints as listed in Table 3.

**Table 3. PET Imaging Biomarkers, Clinical and Biologic Information Database**

PET Imaging Biomarkers <sup>a</sup>	Clinical and Biologic Information
[ <sup>18</sup> F]FLT (proliferation): SUV <sub>max</sub> , SUV <sub>mean</sub> , SUV <sup>FLT</sup> <sub>net</sub> , K <sup>FLT</sup> <sub>net</sub>	Age at diagnosis, performance status, tumor size and location, radiographic characteristics, extent of surgery, dates and types of therapy.
[ <sup>18</sup> F]Fluciclovine amino acid uptake: SUV <sub>max</sub> , SUV <sub>mean</sub> , SUV <sup>FLUC</sup> , K <sup>FLUC</sup> <sub>net</sub>	Tumor histology and WHO grade, vascularity, MIB-1, VEGF, EGFR, p53, IDH status Clinical and radiographic response, concomitant medications; time to progression, overall survival

<sup>a</sup> Quantitative and semi-quantitative imaging biomarkers

The data from multiple PET biomarkers combined with histologic and clinical data will enable identification and development of PET biomarker profiles which correlate with (and might be predictive of) tumor aggressiveness and clinical outcomes. The three imaging time point assessments serve multiple uses in this initial exploratory study. If baseline imaging can predict tumor aggressiveness and clinical outcomes, such imaging may potentially be used to guide selection of conventional, aggressive, or investigational therapies. The second time point, within 1-4 weeks after 6wks of chemoradiotherapy, will provide a measurement of tumor response that may potentially differentiate tumors that will progress rapidly from those that will not (changes in imaging biomarkers between exams 1 and 2 will likely be important for this determination). Finally, the multi-tracer array at the time of treatment effect (pseudoprogression) versus true recurrence/progression may yield new insights into the physiology and biology of these tumors, and multi-tracer imaging may help to differentiate recurrence from radiation necrosis/treatment effect (pseudoprogression). We believe the complementary value of the two tracers in conjunction has much greater potential for meeting these goals than any one tracer alone. For example, tumors with high proliferation ([<sup>18</sup>F]FLT) and/or growth ([<sup>18</sup>F]Fluciclovine) may respond best to anti-EGFR-directed agents or conventional cytotoxic chemotherapies. Of specific interest, is there a multi-tracer PET signature that distinguishes between treatment effect (pseudoprogression) and true progression/recurrence that will have important clinical utility? These questions will be studied in detail, developing formal hypotheses that will be tested in future phase II/III clinical trials.

## 5. TRIAL DESIGN

### 5.1. Patient Eligibility

Two different groups of patients will be able to enroll in the trial. The first group, Group A, will be for patients who have not yet received any therapy for their suspected or newly found brain tumors. Group B will enroll patients with pathologically proven malignant brain tumor (WHO Grade III or IV glial-based tumors) who have undergone chemoradiation and have a contrast enhancing mass ≥ 1.0 cm in greatest diameter concerning for pseudoprogression. MRI-documented possible recurrence/progression versus treatment effect (pseudoprogression) within 6 months from the time of completion of chemoradiation.

Group A will have three different patient cohorts. The first, for patients where there is compelling evidence, based on the MRI and/or CT imaging, that a high-grade primary brain tumor is present. Pathologic confirmation will occur with subsequent biopsy or surgery. The second cohort of patients are individuals where the tumor is felt to be inoperable and a biopsy is performed but no surgery. The third cohort of patients are post-surgery where a complete surgical resection was not possible. This will be confirmed by a post-operative contrast MRI/CT scan where residual tumor  $\geq 1.0$  cm in diameter is present.

Eight to ten patients per year will be enrolled, for a total of 30 patients. Providers within the Radiation Oncology and Neuro-Oncology departments will identify and recruit patients who may be eligible participants.

## 5.2. Inclusion Criteria

- Two adult patient groups will be eligible for inclusion in this study:
  - Group A: Patients where there is compelling evidence, based on the MRI and/or CT imaging, that a high-grade primary brain tumor is present. Pathologic confirmation will occur with biopsy or surgery.  
Patients whose tumor is felt to be inoperable and a biopsy is performed but no surgery.  
Patients with a newly diagnosed primary malignant brain tumor (WHO Grade III or IV) who will be receiving chemoradiation and who either did not undergo surgical resection or underwent incomplete resection with residual tumor  $\geq 1.0$  cm in greatest diameter by contrast MRI postoperatively.
  - Group B: Patients with pathologically proven malignant brain tumor (WHO Grade III or IV glial-based tumors) who have undergone chemoradiation and have MRI-documented possible recurrence/progression versus treatment effect (pseudoprogression) within 6 months from the time of completion of chemoradiation.
- Patients must be 18 years or older for inclusion in this study. There is little experience with the safety of [<sup>18</sup>F]FLT or [<sup>18</sup>F]Fluciclovine in children, and the risks associated with radiation exposure may be increased for children under 18 years old as well.
- Patients must document their willingness to be followed for up to 24 months after recruitment by signing informed consent documenting their agreement to have clinical endpoints and the results of histopathologic tissue analysis (when tissue becomes available from routine care) entered into a research database.
- All patients, or their legal guardians, must sign a written informed consent and HIPAA authorization in accordance with institutional guidelines.
- Determination of pregnancy status: Female patients who are not postmenopausal or surgically sterile will undergo a serum pregnancy test prior to each set of [<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine PET scans. A negative test will be necessary for such patients to undergo research PET imaging.
- Pre-imaging laboratory tests must be performed within 28 days prior to the [<sup>18</sup>F]FLT imaging procedure. These must be less than 4.0 times below or above the upper or lower limit range for the respective laboratory test for entry into the study (unless clinically not relevant). In those instances where a laboratory value is outside of this range, then such a patient will be ineligible for enrollment unless at the discretion of the PI the abnormal lab value is of no clinical significance to the safety or integrity of the study results. For follow-up scanning sessions after therapy has been instituted, laboratory testing will also be required due to the use of [<sup>18</sup>F]FLT. The patients have brain tumors and will have received chemoradiation; therefore, many routine laboratory tests may not be within the typical normal range. As such, a factor of 4.0 times above or below the upper or lower value for the normal range for any laboratory test will also be used to determine the acceptable range for the

2<sup>nd</sup> and possibly 3<sup>rd</sup> imaging time points (unless clinically not relevant as explained above). The baseline laboratory testing will include liver enzymes (ALT, AST), bilirubin (total), serum electrolytes, CBC with platelets, prothrombin time, partial thromboplastin time, BUN, and creatinine. For those patients receiving coumadin or another anticoagulant the upper limit for prothrombin time or partial thromboplastin time must not exceed 6 times the upper limit of the normal range. (Appendix E, Laboratory Study Results).

### **5.3. Exclusion Criteria**

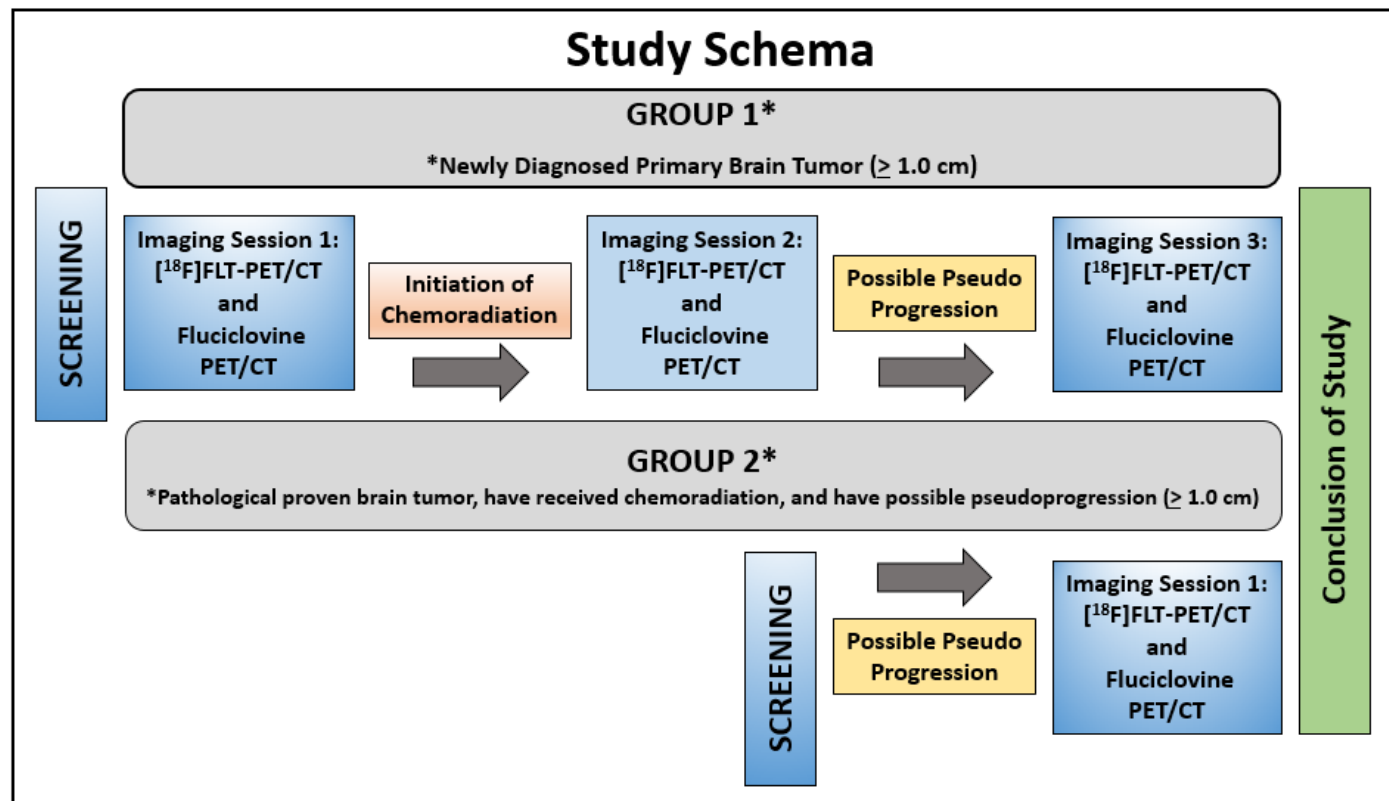
- Patients with known allergic or hypersensitivity reactions to previously administered radiopharmaceuticals. Patients with significant drug or other allergies or autoimmune diseases may be enrolled at the Investigator's discretion.
- Patients who are pregnant or lactating or who suspect they might be pregnant. Serum pregnancy tests will be obtained prior to each set of multi-tracer PET scans in female patients that are not postmenopausal or surgically sterile.
- Adult patients who require monitored anesthesia for PET scanning.
- Patients who are too claustrophobic to undergo PET scanning.
- Known HIV positive patients due to the previous toxicity noted with [<sup>18</sup>F]FLT in this patient group.

### **5.4. Patient Registration**

Following appropriate pre-registration evaluation, a signed consent, HIPAA authorization, and completed eligibility checklist will be obtained (Appendix A). New patient registrations will be submitted to the clinical trials office by the study coordinator, who will record study data on all patients entered into the study and complete subsequent forms.



## 5.5. Study Procedures, Schedule of Events, Tracer Administration: Route and Dosing



### 5.5.1. Initial Visits Prior to PET Imaging

The patient will be under the care of their treating neuro-oncologist. The baseline measures of liver function, renal function, and electrolytes will be collected if not previously obtained within 28 days of the initial [<sup>18</sup>F]FLT imaging visit. The following additional patient data will be obtained: histological diagnosis (when available following surgery), age at radiological diagnosis, gender, and other treatment modalities used. Clinical (non-research related) imaging studies, such as contrast MRI and/or standard clinical FDG PET, will be collected according to standard of care.

### 5.5.2. Multi-Tracer PET Exams

Multi-tracer PET exams of [<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine will be acquired in each patient at up to three time points: (1) prior to any tumor-directed therapy, either prior to surgery or after surgery providing a complete surgical resection was not performed and confirmed by a post-operative contrast MRI scan where residual tumor  $\geq 1.0$  cm in diameter was present and prior to any tumor-directed therapy; (2) within 1-4 weeks after the conclusions of the initial (~6-8 weeks) chemoradiotherapy; and (3) patients with MRI-documented possible recurrence/progression versus treatment effect (pseudoprogression) within 6 months from the time of completion of chemoradiation. The abnormality must be  $\geq 1.0$  cm in diameter by contrast MRI or CT or show changes on non-enhancing MRI sequences (T2 or FLAIR).

Patients who were originally not enrolled to study baseline and conclusion of therapy imaging will be eligible to participate at the time of MRI-documented possible recurrence/progression versus treatment effect (pseudoprogression) within 6 months from the time of completion of chemoradiation. The abnormality must be  $\geq 1.0$  cm in diameter by contrast MRI or CT or show changes on non-enhancing MRI sequences (T2 or FLAIR).

### 5.5.3. Day of Research PET Scan(s)

The patient will have the appropriate IV access placed for radiotracer administration. The patient will then be positioned comfortably on the PET imaging table with the PET head-holder attachment. A CT scan for PET attenuation correction will be acquired using x-ray CT (PET/CT scanner). Dynamic PET scans of each tracer will then be acquired, which requires administration of the PET tracer(s) while the scanner is acquiring in dynamic mode. The details of tracer administration are summarized in Table 4.

Table 4. PET Tracer Administration: Route, Dosing, and Blood Sampling

<i>Tracer</i>	<i>Route</i>	<i>Manner</i>	<i>Injected Dose</i>	<i>Scan Mode</i>
[ <sup>18</sup> F]FLT	IV	~60s push	~10 mCi	Dynamic
[ <sup>18</sup> F]Fluciclovine	IV	~60s push	~10 mCi	Dynamic

Each radiotracer will be prepared by the investigational drug radiochemists in the PET cyclotron facility at HCI on the day of the scanning session. The tracer will be administered to the patient by a physician, nuclear medicine technologist, or trained research personnel in the PET imaging suite. Each tracer administration will be pushed by hand as a manually timed infusion (~60s) as indicated by Table 4, where each infusion is designed to optimize dynamic PET efficacy for that tracer.

Adverse events potentially related to the administration of [<sup>18</sup>F]FLT or [<sup>18</sup>F]Fluciclovine will be recorded at the conclusion of the [<sup>18</sup>F]FLT or [<sup>18</sup>F]Fluciclovine study. (Appendix B: Schedule of Events; Appendix D: [<sup>18</sup>F]FLT Infusion Flow Chart, Appendix G: [<sup>18</sup>F]Fluciclovine Infusion Flow Chart).

## 5.6. Data Collection

### 5.6.1. Safety Data Recorded During [<sup>18</sup>F]FLT-PET and [<sup>18</sup>F]Fluciclovine-PET Scans

Data collected on visit(s) for [<sup>18</sup>F]FLT-PET and [<sup>18</sup>F]Fluciclovine-PET scanning will be recorded on the [<sup>18</sup>F]FLT or [<sup>18</sup>F]Fluciclovine Protocol flow chart and will include: vital signs (including heart rate, blood pressure, and temperature) recorded just prior to the [<sup>18</sup>F]FLT or [<sup>18</sup>F]Fluciclovine infusion and then after the completion of the [<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine imaging study. In addition to monitoring the above-mentioned safety parameters, an emergency code cart is located in the PET suite and can be utilized by the PET imaging team, as well as accessed by the code team at the institution should an emergent situation arise.

Additional information to be collected will include:

- Questioning of patient regarding adverse events immediately after each [<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine administration.
- Patients may be discharged after the completion of the [<sup>18</sup>F]FLT or [<sup>18</sup>F]Fluciclovine imaging procedure if stable and if no adverse events are noted on questioning. Patients will be evaluated for adverse events after the completion of the [<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine scans.

### 5.6.2. Tissue Collection and Analysis

All patients enrolled on this study will have undergone a neurosurgical procedure (either biopsy or tumor resection) in order to obtain a histological diagnosis of their tumors as part of standard care. Tissue specimens will be analyzed by the attending neuropathologist as part of routine care, who will assign a

histologic diagnosis and tumor grade according to World Health Organization (WHO) criteria. If sufficient tissue is available, specimens are routinely evaluated immunohistochemically for MIB-1, EGFR, p53, and IDH status; and by conventional light microscopy for vascularity. These results are routinely recorded on the surgical pathology report.

## 6. METHODS FOR EVALUATION OF IMAGING STUDIES

Each PET imaging study will be evaluated using qualitative, semi-quantitative, or fully-quantitative analysis techniques that have previously been used by us and other groups, and represent the standard and accepted means of evaluating static- and dynamic-PET images for these tracers. These assessments are described below for each tracer.

### 6.1. [<sup>18</sup>F]FLT-PET Imaging

#### 6.1.1. [<sup>18</sup>F]FLT Qualitative Visual Assessment

Qualitative (visual) assessment of level of uptake in brain tumor and normal brain will be performed. Regarding the initial question of tumor presence the following scale will be used: 1= definite tumor, 2 = probably tumor, 3 equivocal for tumor, 4 = probably not tumor, 5 = definitely not tumor. For the immediate post therapy imaging [<sup>18</sup>F]FLT scan the same scales will be used. In the second group of patients where recurrence/progression (pseudoprogression) visual assessment on the [<sup>18</sup>F]FLT-PET study will be interpreted as to confidence of tumor recurrence: 5 = definitely recurrence/progression, 4= probably recurrence/progression, 3= unable to differentiate recurrence/progression, 2= probably treatment effect/necrosis, 1= definitely treatment effect/necrosis.

#### 6.1.2. [<sup>18</sup>F]FLT Semi-Quantitative Assessment

Semi-quantitative assessment of [<sup>18</sup>F]FLT uptake will be performed by calculating  $SUV_{max}$  and  $SUV_{mean}$ . A region-of-interest (ROI) will be drawn over any tumor mass, necrotic region, or other abnormal region(s) on the summed static [<sup>18</sup>F]FLT PET image. For each ROI, standardized uptake values (SUVs) with body surface-area correction will be obtained for both the maximum and mean voxel value in ROI:

$$SUV_{max} = \frac{A_{max}}{InjectedDose/BSA} \quad SUV_{mean} = \frac{A_{mean}}{InjectedDose/BSA}$$

$$BSA = W^{0.425} \times H^{0.725} \times 0.00718$$

$A_{max}$  = maximum voxel value in ROI ( $\mu$ Ci/mL; mCi/L; or MBq/L)

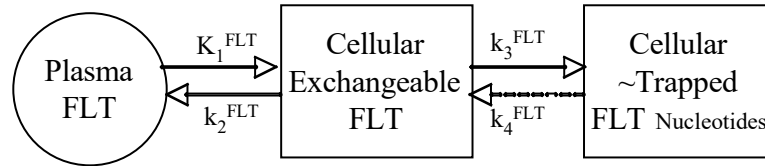
$A_{mean}$  = mean voxel value in ROI ( $\mu$ Ci/mL; mCi/L; or MBq/L)

$BSA$  = body surface area, computed from height H (cm) and weight W (kg)

Ratios of the SUV of tumor to that of white matter (T/WM) and cortex (T/C) will also be computed. The change in SUVs for pre-treatment (baseline) and post-treatment images will be computed and correlated with available histologic and clinical data.

### 6.1.3. [<sup>18</sup>F]FLT Fully-Quantitative Assessment

Quantitative assessment of [<sup>18</sup>F]FLT uptake will be performed by mathematical modeling of time activity curves for [<sup>18</sup>F]FLT and its metabolites in blood and tissues. Several methods of analysis are being considered for evaluating [<sup>18</sup>F]FLT images and results. A plausible scheme is outlined in the figure below.



The compartments describe the kinetic behavior of [<sup>18</sup>F]FLT. Its transport across cell membranes is governed by two rate constants,  $K_1$  and  $k_2$ . The phosphorylation of [<sup>18</sup>F]FLT is governed by the rate constant  $k_3$ . Evidence from FLT experiments in dogs suggests that [<sup>18</sup>F]FLT is retained in cells without significant dephosphorylation ( $k_4$ ). We will initially assume that  $k_4$  is zero to simplify the modeling equations; the Akaike Information Criterion (AIC) will be used to determine if the time-activity curves support the more complex model ( $k_4 \neq 0$ ), which will then be used as indicated.

We will investigate the contribution of the labeled metabolite. If the labeled metabolite is restricted to the plasma space and urine and if the rate constant  $k_4$  is negligible, then the [<sup>18</sup>F]FLT flux constant,  $K_{FLT}$  (mL/min/g), will be computed using graphical analysis (Patlak et al., 1983, Patlak and Blasberg, 1985). In the event that the [<sup>18</sup>F]FLT metabolite contribution in tissue is significant, we will investigate the use of a metabolite-corrected flux constant as a measure of [<sup>18</sup>F]FLT uptake (Mankoff et al., 1997, Muzi et al., 2005a, Muzi et al., 2005b, Shields et al., 2005, Muzi et al., 2006).

The parameters of interest for this model are those directly linked to DNA synthesis—specifically,  $k_3$  and  $K_{flux}$ , as they represent the phosphorylation of [<sup>18</sup>F]FLT to reflect cellular proliferation. We will correlate these rates with proliferative (MIB-1) indices on histological tissue and S phase fraction determined by flow cytometry when these are available.

Procedure for [<sup>18</sup>F]FLT plasma metabolite analyses: Dynamic arterial or heated hand blood sampling will be performed (Phelps et al., 1979). It is anticipated that the majority of patients will be assessed with the heated hand methodology thus eliminating the need for radial artery catheterization. Approximately 23 (1-2 mL) samples will be drawn over a period of 70 minutes with approximately 12 samples drawn in the first 2-3 minutes post injection and additional samples obtained at 4, 5, 7, 10, 15, 20, 30, 45, 60, and 70 minutes after injection. This sampling routine allows for determination of the whole blood and serum time activity curves. The whole blood samples will be counted and then centrifuged to separate plasma from cells. Aliquots of plasma (0.5 mL) will be counted for radioactivity. Another 0.5 mL of each plasma sample will be diluted with 3.5 mL of water and the resulting diluted plasma sample passed sequentially through a QMA cartridge followed by a C18 SepPak brand solid phase extraction cartridge. Then 4 mL of water will be passed sequentially through the cartridges to rinse them. The 8 mL of combined eluents will be counted to measure any [<sup>18</sup>F] breakthrough. The QMA and C18 SepPaks will then be separated. [<sup>18</sup>F]FLT glucuronide conjugate will be eluted from the QMA cartridge by passing 4 mL of 0.25M aqueous sodium citrate through the cartridge. [<sup>18</sup>F]FLT will be eluted by passing 4 mL of ethanol through the C18 SepPak. All of the eluates will be counted for radioactivity. It should be noted that in certain individuals it is not possible to obtain adequate venous access to perform the dynamic blood sampling. In the event that adequate venous access is not possible, this analysis will not be required.

## 6.2. [<sup>18</sup>F]Fluciclovine PET Imaging

### 6.2.1. [<sup>18</sup>F]Fluciclovine Qualitative Visual Assessment

The uptake of [<sup>18</sup>F]Fluciclovine is mediated by the sodium-independent “L” large-neutral amino acid transport (LAT) system. Since amino acid uptake is enhanced in malignancy, it accumulates faster and to a higher degree in cancer cells than normal cells, making it a promising biomarker for cancer diagnosis by PET imaging. Only a small fraction is excreted through the urinary pathway in the first hours after injection and it is not taken up to a high degree by normal brain tissue; therefore, unlike [<sup>18</sup>F] fluorodeoxyglucose FDG, PET imaging with [<sup>18</sup>F]Fluciclovine may be useful for the detection and diagnosis of prostate cancer and malignant gliomas. The ability of [<sup>18</sup>F]Fluciclovine to detect malignant lesions in humans has been assessed in preliminary studies by the Emory group and others. [<sup>18</sup>F]Fluciclovine is reported to have utility in the evaluation of primary and metastatic cancers in the brain, due to its low brain background uptake and high uptake by brain tumors (Shoup et al., 1999, Akhurst et al., 2006, Meirelles et al., 2006).

The following scale will be used to qualitatively assess tracer uptake in areas of abnormality, including tumor mass and necrotic regions, as related to normal brain regions: 1 = definite tumor, 2 = probably tumor, 3 equivocal for tumor, 4 = probably not tumor, 5 = definitely not tumor. In the second group of patients where recurrence/progression (pseudoprogression) visual assessment on the [<sup>18</sup>F]Fluciclovine-PET study will be interpreted as to confidence of tumor recurrence: 5 = definitely recurrence/progression, 4 = probably recurrence/progression, 3 = unable to differentiate recurrence/progression, 2 = probably treatment effect/necrosis, 1 = definitely treatment effect/necrosis.

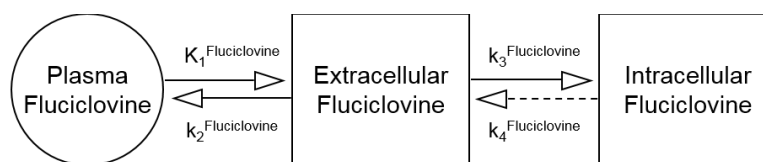
### 6.2.2. [<sup>18</sup>F]Fluciclovine Semi-Quantitative Assessment

Semi-quantitative assessment of [<sup>18</sup>F]Fluciclovine uptake will be performed by calculating  $SUV_{max}$  and  $SUV_{mean}$  in the same manner as for [<sup>18</sup>F]FLT described earlier. SUVs will be calculated for summed static images from 70 minutes post injection. The change in SUVs for pre-treatment (baseline) and post-treatment images will be computed and correlated with available histologic and clinical data.

### 6.2.3. [<sup>18</sup>F]Fluciclovine Fully-Quantitative Assessment

Fully-quantitative compartment modeling analysis of the dynamic [<sup>18</sup>F]Fluciclovine-PET images will be performed according to the two-compartment model shown below, which has been previously described (Sasajima et al., 2013, Sørensen et al., 2013). As with the other tracers, parameters of interest include individual rate parameters ( $K_1$ ,  $k_2$ - $k_4$ ) and the net uptake parameters. The primary imaging endpoints for [<sup>18</sup>F]Fluciclovine are not yet fully understood, and will in part be elucidated by this study. Of interest, the second-order retention ( $k_3$ ) and reversible ( $k_4$ ) parameters for this tracer have high potential for differentiating inflammatory processes occurring after radiotherapy from residual tumor activity.

Compartment modeling techniques will be applied to the dynamic PET data to obtain estimates of kinetic rate parameters ( $K_1$ ,  $k_2$ - $k_4$ ) and FDG net uptake ( $K_{net}$ ). Individual rate parameters will be computed using full compartment-modeling techniques according to the two-tissue compartment model shown below. Commercially available PET analysis software (e.g. PMOD Technologies LTD., Zurich, Switzerland) will be used. The net uptake parameter will be computed from both full compartment modeling and Patlak graphical analysis (Patlak et al., 1983, Patlak and Blasberg, 1985), both of which are standard and accepted methods.



Procedure for [<sup>18</sup>F]Fluciclovine plasma analyses: Dynamic arterial or heated hand blood sampling will be performed (Phelps et al., 1979). It is anticipated that the majority of patients will be assessed with the heated hand methodology thus eliminating the need for radial artery catheterization. Approximately 23 (1-2 mL) samples will be drawn over a period of 70 minutes with approximately 12 samples drawn in the first 2-3 minutes post injection and additional samples obtained at 4, 5, 7, 10, 15, 20, 30, 45, 60, and 70 minutes after injection. This sampling routine allows for determination of the whole blood and serum time activity curves. The whole blood samples will be counted and then centrifuged to separate plasma from cells. Aliquots of plasma (0.5 mL) will be counted for radioactivity. It should be noted that in certain individuals it is not possible to obtain adequate venous access to perform the dynamic blood sampling. In the event that adequate venous access is not possible, this analysis will not be required.

### 6.3. Multi-Tracer Assessments in Conjunction

The exploratory aims of this study will assess the value of multi-tracer imaging endpoints, used in conjunction, over individual PET tracers for tumor characterization, predicting tumor aggressiveness, determination of functional changes that occur in response to therapy, and differentiating between recurrent/progressive tumor and treatment effect/necrosis (pseudoprogression). All three phases of image assessment will be studied: qualitative visual assessment (e.g. image scoring), semi-quantitative measures of tracer uptake (SUVs), and fully-quantitative measures ( $K_1$ - $k_4$ ;  $K_{net}$ ). In each case, combinations of imaging endpoints will be considered in conjunction, such as SUV ratios of tracer pairs, and compared with histologic measurements and clinical outcomes.

## 7. DATA ANALYSIS AND STATISTICS

### 7.1. Validation of Multi-Tracer PET Imaging Technology

This study will explore several hypotheses and provide preliminary exploratory data in the value of the two PET tracers [<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine alone and in conjunction to assess response and differentiate tumor recurrence/progression from treatment effect/necrosis (pseudoprogression). The broader question of validating precisely what physiological quantity or process that each tracer/imaging endpoint measures and how these correlate with clinical endpoints and histologic data, when available, is the purpose of the study.

### 7.2 Data Analysis

*Qualitative and Visual Assessment.* The static images for each tracer will be visually assessed for any artifacts. The images will then be scored as described earlier for each tracer based on the level of uptake and confidence of tumor presence. Bland-Altman plots will be prepared.

*Semi- and Fully-Quantitative Assessments.*

#### Primary Endpoint

The primary endpoint of the study is that a 25% reduction in  $SUV_{max}$  or  $SUV_{mean}$  for each of the PET imaging tracers will be predictive of increased overall survival. These endpoints will be analyzed by

Kaplan-Meier analysis and logrank tests. Cox proportional hazards regression will also be used to estimate hazard ratios. Each analysis will be performed at the two sided 0.0125 significance level to adjust for four comparisons.

### Secondary Exploratory Objectives

No multiple comparison adjustment will be performed for analysis of secondary endpoints.

- Assess the association between overall survival and change in each of the following quantitative markers for [<sup>18</sup>F]Fluciclovine and [<sup>18</sup>F]FLT:  $K_1-k_4$ ,  $K_{net}$  and  $K^{trans}$ , from baseline to post therapy. This association will be addressed by Kaplan-Meier curves and associated logrank tests. Cox proportion hazards regression will be used to estimate hazard ratios.
- Assess the association between progression free survival and change in each of the following quantitative markers for [18F]Fluciclovine and [18F]FLT:  $K_1-k_4$ ,  $K_{net}$  and  $K_{trans}$ , from baseline to post therapy. This association will be addressed by Kaplan-Meier curves and associated logrank tests. Cox proportion hazards regression will be used to estimate hazard ratios.
- Assess the ability of [<sup>18</sup>F]FLT and/or [<sup>18</sup>F]Fluciclovine alone or together to predict true progression from pseudoprogression in patients with this clinical dilemma. True progression will be separated from pseudoprogression by subsequent MRI imaging. Receiver operating characteristic curve (ROC) analysis with true progression as outcome and each imaging marker or best combination as predictor will be used for this assessment. Bootstrap methods will be used to assess the standard error.
- A 25% reduction in  $SUV_{max}$  or  $SUV_{mean}$  for each of the PET imaging tracers will be more predictive of longer overall survival, than the current MRI based response criteria. This endpoint will be addressed by informal comparison of Kaplan-Meier curves, log rank p-values, and hazard ratios from separate analyses of overall survival for each imaging parameter.
- A 25% reduction in  $SUV_{max}$  or  $SUV_{mean}$  for each of the PET imaging tracers will be more predictive of time to progression than the current MRI based response criteria. This endpoint will be addressed by informal comparison of Kaplan-Meier curves, log rank p-values, and hazard ratios from separate analyses of time to progression for each imaging parameter.

### 7.3. Justification of Sample Size

(Sample size calculations were prepared by Kenneth Boucher, Ph.D. of the HCI Biostatistics Resource)

Our justification of sample size is based on the data from Chen et al and Schwarzenberg et al. (Chen et al., 2007, Schwarzenberg et al., 2012) in which a more than 25% reduction in tumor [<sup>18</sup>F]FLT uptake as measured by standardized uptake value was defined as a metabolic response. In this study there were nine responders (47%) and 10 non-responders (53%). Responders survived three times as long as non-responders (10.8 v 3.4 months;  $P = .003$ ), and tended to have a prolonged progression-free survival ( $P = .061$ ). Both early and later [<sup>18</sup>F]FLT -PET responses were more significant predictors of overall survival (1 to 2 weeks,  $P = .006$ ; 6 weeks,  $P = .002$ ), compared with the MRI responses ( $P = .060$  for both 6-week and best responses). A potential limitation of this study was the use of standardized uptake value rather than full kinetic analysis in brain tumors (Peck et al., 2015). We will use full kinetic modelling and hopefully provide even more robust response data for [<sup>18</sup>F]FLT. Since there are no human studies utilizing [<sup>18</sup>F]Fluciclovine to measure response we have extrapolated from the available human xenograft rat glioma study of Ono et al. (2015b) utilizing anti-<sup>14</sup>C-FACBC with U87 and U87R (TMZ-resistant subculture) cells which were inoculated into the right and left basal ganglia. In autoradiographic experiments the DAR of anti-<sup>14</sup>C-FACBC was significantly higher in tumors than in non-involved cortices. <sup>14</sup>C-FACBC accumulation was lower after single-agent treatment and lower still after combination therapy. Treatment with temozolomide

alone significantly decreased the T/NT for the DAR of U87 tumors, whereas the T/NT ratios for those of U87R tumors remained unchanged. Temozolomide and interferon- $\beta$  combination treatment significantly decreased anti-<sup>14</sup>C-FACBC uptake in both U87R and U87 tumors. Addition of bevacizumab to the temozolomide and interferon- $\beta$  combination further decreased anti-<sup>14</sup>C-FACBC uptake in both tumor types.

With an initial sample size of 30, we estimate that approximately 75% of the subjects (N = 22) will have both a [<sup>18</sup>F]Fluciclovine-PET and [<sup>18</sup>F]FLT-PET after completion of initial chemoradiotherapy. Our justification of sample size is based on data on the prediction of OS using PET reported in Chen et al. (Chen et al., 2007). In this study more than 25% reduction in tumor [<sup>18</sup>F]FLT uptake as measured by standardized uptake value was defined as a metabolic response. In this study there were nine responders (47%) and 10 non-responders (53%). Responders survived three times as long as non-responders (10.8 v 3.4 months;  $P = .003$ ), and tended to have a prolonged progression-free survival ( $P = .061$ ). Both early and later [<sup>18</sup>F]FLT - PET responses were more significant predictors of overall survival (1 to 2 weeks,  $P = .006$ ; 6 weeks,  $P = .002$ ), compared with the MRI responses ( $P = .060$  for both 6-week and best responses). In univariate and multivariate Cox regression analysis, lack of FLT reduction at 6 weeks was the best predictor of reduced OS, with adjusted hazard ratio = 4.955 ( $p = 0.02$ ). Assuming 50% of subjects are metabolic responders, median survival of 3.4 months in non-responders and two years of follow up, a sample size of N = 22 subjects with follow up PET scans will provide 85% power to detect a hazard ratio of 4.9 using a two-sided logrank test at the 0.0125 significance level.

## 8. REGULATORY AND REPORTING REQUIREMENTS

The AE reporting time frame for this study with two investigational radiopharmaceuticals is 24 hours post injection of each tracer. This is defined in FDA regulation (see section 8.4.1). Per the approval of IND 76, 843 for [<sup>18</sup>F]FLT on 2/1/07 the following reporting of unexpected fatal or life threatening events, serious adverse events, and serious and unexpected adverse events will occur: (1) Reporting any unexpected fatal or life threatening adverse experience associated with the use of [<sup>18</sup>F]FLT by telephone or fax no later than 7 calendar days after initial receipt of the information. (2) Reporting any adverse experience associated with the use of [<sup>18</sup>F]FLT, that is both serious (SAE) and unexpected in writing no later than 15 calendar days after initial receipt of the information. (3) Submitting annual reports. The reportable events will also be submitted to the IRB using the University of Utah ERICA online system: <https://erica.research.utah.edu/erica/Rooms/DisplayPages/LayoutInitial?Container=com.webbridge.entity.Entity%5BBOID%5B5FD2DA60262617429607E459C0E09D92%5D%5D>

The same reporting process and requirements will be used for [<sup>18</sup>F]Fluciclovine.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 with subsequent modifications will be utilized for adverse event reporting (<http://ctep.cancer.gov/reporting/index.html>).

All appropriate treatment areas will have access to a copy of the CTCAE version 5.0 with modifications. A list of adverse events that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in Section 6 (Methods for Evaluation of Imaging Studies).

### 8.1. Informed Consent

Informed consent will be obtained from all research participants prior to performing any study procedures using the most recent IRB approved version.



## 8.2 Institutional Review

Study will be approved by the Institutional Review Board of University of Utah.

## 8.3 Data and Safety Monitoring Plan

A Data and Safety Monitoring Committee (DSMC) is established at Huntsman Cancer Institute (HCI) and approved by the NCI to assure the well-being of patients enrolled on Investigator Initiated Trials that do not have an outside monitoring review. Roles and responsibilities of the DSMC are set forth in the NCI approved plan. The activities of this committee include a quarterly review of adverse events including SAEs, important medical events, significant revisions or amendments to the protocol, and approval of cohort/dose escalations. If the DSMC and/or the PI have concerns about unexpected safety issues, the study will be stopped and will not be resumed until the issues are resolved. The DSMC also reviews and approves audit reports generated by the Research Compliance Office.

### 8.3.1 Data Reporting

This study will be monitored by the Principal Investigator using the ERICA system. In addition, the study will be monitored by the HCI Data and Safety Monitoring Committee. Cumulative data will be submitted electronically to ERICA, the HCI Data and Safety Monitoring Committee, and the IRB as required.

## 8.4 Adverse Events / Serious Adverse Events

This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) version 5.0 for AE and SAE reporting. An electronic copy of the CTCAE version 5.0 can be downloaded from: [https://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/etc.htm#etc\\_50](https://ctep.cancer.gov/protocolDevelopment/electronic_applications/etc.htm#etc_50)

### 8.4.1 Adverse Events (AE)

An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after receiving the radioactive tracer(s). For the purposes of this study, the terms toxicity and adverse event are used interchangeably. Medical conditions/diseases present before starting the study are only considered adverse events if they worsen after being injected with the radioactive tracer(s). Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

Adverse event reporting for studies assessing diagnostic radiopharmaceuticals have different criteria than for therapeutic drugs. The NCI CTEP requires that AE's be reported for up to ten half-lives after the administration of the tracer rounded UP to the nearest whole day. In this instance, the half-life of the <sup>18</sup>F radioisotope is 110 minutes, with ten half-lives equivalent to 18.33 hours. This study will therefore collect adverse events from the time the radiopharmaceutical is administered and 24 hours afterward, even if the event is not considered to be related to the tracer.

The collection of any adverse events will begin when a patient receives their first dose of [<sup>18</sup>F]FLT or [<sup>18</sup>F]Fluciclovine and will end 24 hours after receiving that dose. As there is the potential for each patient to receive up to six scanning visits, each constituting a radioactive tracer to be injected into the patient, the adverse events will be monitored for 24 hours after each injection and subsequent scan. Those adverse events that are not associated with [<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine that do not require expedited reporting will be reported in the routine manner to the IRB.

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit or phone contact during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits within the AE reporting period or through physical examination, laboratory tests, or other assessments. As far as possible, each adverse event should be evaluated to determine:

1. The severity grade based on CTCAE v.5 (Grade 1-5).
2. Its relationship to the study radioactive tracer(s) (definite, probably, possible, unlikely, not related)
3. Its duration (start and end dates or if continuing at final exam).
4. Action taken (no action taken; study tracer dosage adjusted/temporarily interrupted; study tracer permanently discontinued due to this adverse event; concomitant medication taken; non-drug therapy given; hospitalization/prolonged hospitalization).
5. Whether it constitutes an SAE.

All adverse events will be treated appropriately. Once an adverse event is detected, it should be followed until its resolution, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study tracer, the interventions required to treat it, and the outcome.

Information about common side effects already known about the tracer is described in the Pharmacology and Safety of [<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine and Radiation Dosimetry of [<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine (Section 3). This information will be included in the patient informed consent and will be discussed with the patient during the study as needed.

All adverse events will be immediately recorded in the patient research chart.

#### 8.4.2 Serious Adverse Event (SAE)

Information about all serious adverse events within the reporting period will be collected and recorded. A serious adverse event is an undesirable sign, symptom, or medical condition which:

- Is fatal or life-threatening.
- Results in persistent or significant disability/incapacity.
- Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above.
- Causes congenital anomaly or birth defect.
- Requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
  - Routine treatment or monitoring of the studies indication, not associated with any deterioration in condition (procedures such as central line placements, paracentesis, pain control).
  - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug.
  - Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission.
  - Social reasons and respite care in the absence of any deterioration in the patient's general condition.

**Note: All deaths on study during the AE reporting window will be reported using expedited reporting regardless of causality. Attribution to treatment or other cause will be provided.**

Fatal and life-threatening events will be reported to the IRB within 24 hours of notification of the event, indicating that a full report will follow. Any unexpected fatal or life threatening adverse event associated with the use of [<sup>18</sup>F]FLT or [<sup>18</sup>F]Fluciclovine will be reported to the FDA by telephone or fax no later than 7 calendar days after initial receipt of the information. All reportable adverse events will be submitted to the FDA & IRB within the required timeframe by as mandated by the FDA and IRB.

Toxicities which fall within the definitions listed above must be reported as an SAE regardless if they are felt to be related to the radioactive tracer(s) or not. Toxicities unrelated to the radioactive tracer(s) that do NOT fall within the definitions above, must simply be documented as AEs in the patient research chart.

## 8.5 SAE Reporting Requirements

SAEs must be reported to the DSMC, the FDA, the IRB and Blue Earth Diagnostics, according to the requirements described below:

A MedWatch 3500 A form must be completed and submitted to [compliance@hci.utah.edu](mailto:compliance@hci.utah.edu) as soon as possible, but no later than 10 days of first knowledge or notification of event (5 days for fatal or life threatening event).

\*MedWatch 3500A form can be found at:

<http://www.fda.gov/downloads/Safety/MedWatch/HowToReport/DownloadForms/ucm082728.pdf>

DSMC Notifications:

- An HCI Research Compliance Officer (RCO) will process and submit the MedWatch form to the proper DSMC member as necessary for each individual study.
- The RCO will summarize and present all reported SAEs according to the Data and Safety Monitoring Plan at the quarterly DSMC meeting.

FDA Notifications:

- Adverse events occurring during the AE reporting period of a clinical study that meet the following criteria will be promptly reported to the FDA:
  - Serious
  - Unexpected
  - Definitely, Probably, or Possibly Related to the investigational drug
  - Fatal or life-threatening events that meet the criteria above will be reported within 7 calendar days after first knowledge of the event by the investigator; followed by as complete a report as possible within 8 additional calendar days.
  - All other events that meet the criteria above will be reported within 15 calendar days after first knowledge of the event by the investigator.
  - The RCO will review the MedWatch report for completeness, accuracy and applicability to the regulatory reporting requirements.
  - The RCO will ensure the complete, accurate and timely reporting of the event to the FDA.
  - The Regulatory Coordinator will submit the report as an amendment to the IND application.
  - All other adverse events and safety information not requiring expedited reporting that occur or are collected during the course of the study will be summarized and reported to the FDA through the IND Annual Report.

IRB Notification:

- Events meeting the University of Utah IRB reporting requirements (<http://www.research.utah.edu/irb/>) will be submitted through the IRB’s electronic reporting system within 10 working days.

**Sponsor/Drug Manufacturer Notifications:**

- All SAEs within the 24 hour reporting period following [<sup>18</sup>F]Fluciclovine PET/CT must be reported within 24 hours of the Site Study Team becoming aware of the event. Contact information for the study team is as follows:
  - Blue Earth Diagnostics  
SAE Email: [Drugsafety@pharsafer.com](mailto:Drugsafety@pharsafer.com)  
Tel: 1-855 AXUMIN1 (1 855 298 6461)  
Fax: +44 (0) 1483 212178

**8.6 Expedited Adverse Event Reporting**

8.6.1 Expedited Reporting Guidelines – Phase 1/2 studies with Investigational Agents

The guidelines given in Table 5 below will be followed. Hospitalization will be defined as any medical event equivalent to CTCAE (Version 5) grade 3, 4, or 5 that precipitated hospitalization (or prolongation of existing hospitalization).

**Table 5.**

UNEXPECTED EVENT		EXPECTED EVENT	
GRADES 2 – 3 Attribution of Possible, Probable or Definite	GRADES 4 and 5 Regardless of Attribution	GRADES 1 - 3	GRADES 4 and 5 Regardless of Attribution
Expedited report within 7 working days.  (Grade 1 Adverse Event Expedited Reporting NOT required.)	Report by phone to IRB within 24 hrs. Expedited report to follow within 10 working days.	Adverse Event Expedited Reporting NOT required.	Expedited report, including Grade 5

For **Hospitalization** only – Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitated hospitalization (or prolongation of existing hospitalization) must be reported regardless of designation as expected or unexpected and attribution.

**8.7 Protocol Amendments**

Any amendments or administrative changes in the research protocol during the period, for which the IRB approval has already been given, will not be initiated without submission of an amendment for IRB review and approval.

These requirements for approval will in no way prevent any immediate action from being taken by the investigator in the interests of preserving the safety of all patients included in the trial.

Any amendments to the protocol that significantly affect the safety of subjects, scope of the investigation, or the scientific quality of study are required to submit the amendment for FDA review.

## 8.8 Protocol Deviations

A protocol deviation (or violation) is any departure from the defined procedures and treatment plans as outlined in the protocol version submitted and previously approved by the IRB. Protocol deviations have the potential to place participants at risk and can also undermine the scientific integrity of the study thus jeopardizing the justification for the research. Protocol deviations are unplanned and unintentional events. The inability to obtain the dynamic blood sampling for [<sup>18</sup>F]FLT and/or [<sup>18</sup>F]Fluciclovine will not be considered a protocol deviation.

Because some protocol deviations pose no conceivable threat to participant safety or scientific integrity, reporting is left to the discretion of the PI within the context of the guidelines below. The IRB requires the **prompt reporting** of protocol deviations which are:

- Exceptions to eligibility criteria.
- Intended to eliminate apparent immediate hazard to a research participant or
- Harmful (caused harm to participants or others, or place them at increased risk of harm – including physical, psychological, economic, or social harm), or
- Possible serious or continued noncompliance.

## 8.9 FDA Annual Reporting

An annual progress report will be submitted to the FDA within 60 days of the anniversary of the date that the IND went into effect. (21 CFR 312.22).

## 8.10 Clinical Trials Data Bank

The study will be registered on <http://clinicaltrials.gov> and the NCI CTRG (Clinical Trials Reporting Program) by the Clinical Trials Office.

## 8.11 Record Keeping

Per 21 CFR 312.57, Investigator records shall be maintained for a period of 2 years following the date a marketing application is approved; or, if no application is filed or the application is not approved, until 2 years after the investigation is discontinued and the FDA is notified.

# 9. PET TRACER PRODUCTION

## 9.1. [<sup>18</sup>F]FLT

### 9.1.1. Study Agent

This protocol utilizes 3'-deoxy-3'-[<sup>18</sup>F]fluorothymidine, [<sup>18</sup>F]FLT, as an imaging agent for measuring cellular proliferation in primary high-grade brain tumors. The agent is administered by intravenous injection

of ≤10 mL solution of 0.1 mCi/kg, up to approximately 10 mCi maximum, of [<sup>18</sup>F]FLT. The non-radioactive [F-19]FLT in the injectate will be ≤6.1 μg (25 nmol). The [<sup>18</sup>F]FLT is formulated in a solution of 92% 0.01 M phosphate buffered saline (PBS), USP with 8% ethanol, USP (v:v). The injectate may be diluted with isotonic saline for injection, USP.

### 9.1.2. Reported Adverse Events and Potential Risks

No adverse events have been reported for [<sup>18</sup>F]FLT at the dosage described for this study. As described in part 3.2 of this document, non-radioactive FLT has been investigated as an anti-AIDS drug and some adverse effects, namely a reversible peripheral neuropathy, were observed in subjects exposed to 50 ng-h/mL plasma over a course of 16 weeks. The dose anticipated for this study will be nominally 1 ng-h/mL for a single injection. The radiation exposure associated with this study is described in part 3.5 of this document and is comparable to the dose for other widely used clinical nuclear medicine procedures.

### 9.1.3. Production of the Radiopharmaceutical

The 3'-deoxy-3'-[<sup>18</sup>F]fluorothymidine [<sup>18</sup>F]FLT used in this study will be prepared locally by the PET Radiochemistry Group at the University of Utah. The precursors for the radiosynthesis include F-18 prepared at the Huntsman Cancer Institute cyclotron from proton irradiation of [O-18]water and an organic precursor, 5'-O-Benzoyl-2, 3'-anhydrothymidine, supplied by ABX. The only other reagents used in the synthesis are potassium carbonate, water, dimethyl sulfoxide, acetonitrile, kryptofix [2.2.2] (a phase transfer agent), USP absolute ethanol, USP PBS, USP sterile water for injection, and USP saline for injection. The radiopharmaceutical product is a clear and colorless liquid that is stored at room temperature in a sterile serum vial. The [<sup>18</sup>F]FLT has an expiration time of eight hours after sterile filtration or when the Ci/mmol or mCi/mL fall below the specified limits.

### 9.1.4. Agent Accountability

[<sup>18</sup>F]FLT is a radiopharmaceutical produced in the cyclotron facility at the Huntsman Cancer Institute. The agent is investigational and approved by the FDA under IND # 76,843. The organic precursor for [<sup>18</sup>F]FLT is provided by ABX in Germany. The precursor is stored in a controlled temperature refrigerator in a locked and secure room and is inventoried with a chain of custody maintained from the time of receipt. Each radiosynthesis is done by University of Utah cyclotron and radiochemistry staff and the product [<sup>18</sup>F]FLT in a dose calibrated syringe will be released after passing all required quality control assays to the physician who will be responsible for administering the appropriate amount (Jeffrey Yap, PhD or his designee). The quality control tests that must be passed prior to release of the product [<sup>18</sup>F]FLT for injection include the radioactive purity, the radiochemical purity, sterilizing filter integrity, tests for kryptofix, DMSO, acetonitrile, pyrogens and particulates. The [<sup>18</sup>F]FLT dose is drawn up into a syringe, assayed for mCi at the time of injection, and administered to the research subject.

## **9.2. [<sup>18</sup>F]Fluciclovine**

### 9.2.1. Study Agent

[<sup>18</sup>F]Fluciclovine is a fluorine-18 labelled PET diagnostic agent supplied as a ready-to-inject solution in either vials or syringes. The maximum dose volume is 5 ml. The drug substance is [<sup>18</sup>F]Fluciclovine. Presence of the *syn*-isomer is minimized in the production process. The formulation of [<sup>18</sup>F]Fluciclovine drug product contains trisodium citrate buffer in water. The pH of the drug product is 3.5-6.0. The radiochemical purity (RCP) is greater than 95% throughout the shelf-life (up to 8 hours). The pH is kept low to avoid the risk of degradation of [<sup>18</sup>F]Fluciclovine. [<sup>18</sup>F]Fluciclovine injection is manufactured by

automated radiosynthesis followed by formulation with buffer and aseptic dispensing in a remotely controlled system. Fluorine-18 decays by positron emission ( $\beta^+$  decay, 96.7%) and orbital electron capture (3.3%) with a half-life of approximately 110 minutes (mins). The positron undergoes annihilation with an electron to produce two gamma photons each of energy 511 keV (193.4% emission).

[<sup>18</sup>F]Fluciclovine injection is a sterile, aqueous solution of [<sup>18</sup>F]Fluciclovine and excipients for intravenous administration. The product is supplied with a radioactive content of 200 MBq/ml (5.4 mCi/ml) at the reference date and time in a syringe or a glass vial sealed with a synthetic rubber closure and aluminum overseal and then withdrawn into syringes at the clinical site. Each vial or syringe is transported in a lead or tungsten shield. The quality control (QC) analysis of a sample of the drug product may be performed in parallel with transportation of the drug product to the study site. The investigator (or nominated deputy) will receive release/reject information for the drug product. Only product for which confirmation of release has been received shall be used. Where the product is transported as a single patient dose, the dose will be measured in a dose calibrator before administration. Where the product is transported in its original container the volume of injection for each patient is calculated and withdrawn into a shielded syringe immediately before injection. The calculation is based on the radioactive content, the half-life of fluorine-18 (109.8 mins), the reference date and time, the prescribed dose and the time of injection. Each patient dose will contain up to approximately 370 MBq (10.0 mCi) at the time of administration. The doses will contain no more than 170  $\mu$ g/ml [<sup>18</sup>F]Fluciclovine and related substances. The maximum administered dose volume is 5 ml.

#### 9.2.2. Reported Adverse Events and Potential Risks

No serious adverse reactions have been reported from any investigator sponsored studies or from the compassionate use program in Norway as described in part 3.1.4 of this document. The risks to subjects mainly relate to the intravenous injection and intravenous blood sampling procedures, and the radiation emitted by [<sup>18</sup>F]Fluciclovine. Intravenous injection and the use of an intravenous cannula are known to carry a small risk of infection and hematoma. The exposure to radiation will not exceed that which is considered acceptable in accordance with appropriate guidelines.

#### 9.2.3. Production of the Radiopharmaceutical

The [<sup>18</sup>F]Fluciclovine used in this study will be prepared locally by the PET Radiochemistry Group at the University of Utah. The precursors for the radiosynthesis include F-18 prepared at the Huntsman Cancer Institute cyclotron from proton irradiation of [O-18]water and an organic precursor supplied in the cassette to be used on the GE FastLab synthesis module. [<sup>18</sup>F]Fluciclovine is manufactured by automated radiosynthesis on the GE FastLab followed by formulation with buffer and aseptic dispensing in a remotely controlled system. The formulation of [<sup>18</sup>F]Fluciclovine drug product contains trisodium citrate buffer in water. The pH of the drug product is 3.5-6.0. The radiochemical purity (RCP) is greater than 95% throughout the shelf-life (up to 8 hours). The pH is kept low to avoid the risk of degradation of [<sup>18</sup>F]Fluciclovine. The radiopharmaceutical product is a clear and colorless liquid that is stored at room temperature in a sterile serum vial. The [<sup>18</sup>F]Fluciclovine has an expiration time of eight hours after sterile filtration.

#### 9.2.4. Agent Accountability

[<sup>18</sup>F]Fluciclovine is a radiopharmaceutical produced in the cyclotron facility at the Huntsman Cancer Institute. The agent is investigational and approved by the FDA under a pending IND.

The shelf-life of [<sup>18</sup>F]Fluciclovine is up to 10 hours from the end of production and the product must not be used beyond this limit. [<sup>18</sup>F]Fluciclovine should be stored at 15-25°C in a shielded container. All non-radioactive containers (shielding, transport cans) must be returned to the manufacturing site. Containers that are radioactive or that contained radioactive products must be destroyed at either the study site or another designated facility, after the study and after overall drug accountability has been completed by the sponsor or its representative. Waste must be disposed of according to national regulations for radioactive material. Precautions for the safe handling of radioactive materials should be observed.

Each radiosynthesis is done by University of Utah cyclotron and radiochemistry staff and the product [<sup>18</sup>F]Fluciclovine in a dose calibrated syringe will be released after passing all required quality control assays to the physician who will be responsible for administering the appropriate amount (Jeffrey Yap, PhD or his designee). The quality control tests that must be passed prior to release of the product [<sup>18</sup>F]Fluciclovine for injection include the radioactive purity, the radiochemical purity, sterilizing filter integrity, tests impurities, pyrogens and particulates. The [<sup>18</sup>F]Fluciclovine dose is drawn up into a syringe, assayed for mCi at the time of injection, and administered to the research subject.



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## **11. APPENDICES**

### APPENDIX A

Eligibility Check List

### APPENDIX B

Schedule of Events

### APPENDIX C

[<sup>18</sup>F]FLT Infusion Flow Chart for Staff

### APPENDIX D

[<sup>18</sup>F]Fluciclovine Infusion Flow Chart for Staff

### APPENDIX E

[<sup>18</sup>F]FLT Patient Adverse Event Questionnaire

### APPENDIX F

[<sup>18</sup>F]Fluciclovine Patient Adverse Event Questionnaire

### APPENDIX G

NCI Common Toxicity Criteria

## APPENDIX A: Eligibility Check List

**Patient Name:** \_\_\_\_\_ **MRN:** \_\_\_\_\_

**Person Confirming Eligibility:** \_\_\_\_\_ **Date:** \_\_\_\_\_

\_\_\_\_\_ (Yes/No) Patient is  $\geq$  18 years old.

\_\_\_\_\_ (Yes/No) Group A. Either patients where there is compelling evidence, based on the MRI and/or CT imaging, that a high-grade primary brain tumor is present. Pathologic confirmation will occur with biopsy or surgery. OR patients whose tumor is felt to be inoperable and a biopsy is performed but no surgery. OR patients with a newly diagnosed primary malignant brain tumor (WHO Grade III or IV) who will be receiving chemoradiation and who either did not undergo surgical resection or underwent incomplete resection with residual tumor  $\geq$  1.0 cm in greatest diameter by contrast MRI postoperatively.

\_\_\_\_\_ (Yes/No) Group B. Patients with pathologically proven malignant brain tumor (WHO Grade III or IV glial based tumors) who have undergone chemoradiation and have MRI-documented (contrast enhancing mass  $\geq$  1.0 cm in greatest diameter) possible recurrence/progression versus treatment effect (pseudoprogession) within 6 months from the time of completion of chemoradiation.

\_\_\_\_\_ (Yes/No) Patient is willing to have their clinical records reviewed for at least 24 months after enrollment.

\_\_\_\_\_ (Yes/No) Patient, or their legal guardians, have signed a written informed consent and HIPAA authorization in accordance with institutional guidelines.

\_\_\_\_\_ (Yes/No) If patient is female, she must be postmenopausal for a minimum of one year, surgically sterile, or is willing to undergo a serum pregnancy test prior to each imaging session.

\_\_\_\_\_ (Yes/No) Patient is not lactating.

\_\_\_\_\_ (Yes/No) Pre- [<sup>18</sup>F]Fluciclovine and [<sup>18</sup>F]FLT PET laboratory tests not greater than 4.0 $\times$  the upper or lower values in the normal range: liver enzymes (AST, ALT, ALK), bilirubin (total), serum electrolytes, CBC with platelets, prothrombin time, partial thromboplastin time, BUN, and creatinine. For those patients receiving coumadin or another anticoagulant the upper limit for prothrombin time or partial thromboplastin time must not exceed 6 times the upper limit of the normal range.

\_\_\_\_\_ (Yes/No) Patient does not have allergic or hypersensitivity reactions to previously administered radiopharmaceuticals.

\_\_\_\_\_ (Yes/No) Patient does not require monitored anesthesia for PET/CT imaging.

\_\_\_\_\_ (Yes/No) Patient is not too claustrophobic to undergo PET/CT imaging

\_\_\_\_\_ (Yes/No) Patient is NOT known to be HIV positive.

Completed by \_\_\_\_\_ Date: \_\_\_\_\_

Physician Verification \_\_\_\_\_ Date: \_\_\_\_\_

3<sup>rd</sup> Party Verification: \_\_\_\_\_ Date: \_\_\_\_\_

## APPENDIX B: Schedule of Events

	Screen	Imaging Session 1		Imaging Session 2		Imaging Session 3	
		[ <sup>18</sup> F]FLT	[ <sup>18</sup> F]Fluciclovine	[ <sup>18</sup> F]FLT	[ <sup>18</sup> F]Fluciclovine	[ <sup>18</sup> F]FLT	[ <sup>18</sup> F]Fluciclovine
<b>Assessment/ Procedures</b>							
Informed Consent	X						
Inclusion/ Exclusion Criteria	X						
Laboratory Monitoring	X <sup>1,2</sup>			X <sup>1,3</sup>		X <sup>1,4,7</sup>	
Infusion of [ <sup>18</sup> F]FLT		X		X		X	
[ <sup>18</sup> F]FLT PET/CT Imaging		X		X		X	
Infusion of [ <sup>18</sup> F]Fluciclovine			X		X		X
[ <sup>18</sup> F]Fluciclovine PET/CT Imaging			X		X		X
Vital Signs <sup>5</sup>		X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>	X <sup>5</sup>
AE Assessment		X	X	X	X	X	X
Concomitant Medication		X <sup>6</sup>		X <sup>6</sup>		X <sup>6</sup>	

- Laboratory tests will include: (AST, ALT), bilirubin (total), serum electrolytes, CBC with platelets, prothrombin time, partial thromboplastin time, BUN, and creatinine. Female patients who are not postmenopausal or surgically sterile will undergo a serum pregnancy test prior to each imaging session ([<sup>18</sup>F]FLT and [<sup>18</sup>F]Fluciclovine PET scans). The values for the imaging sessions must be within 4.0 X the upper or lower normal limits (unless clinically not relevant). For those patients receiving coumadin or another anticoagulant the upper limit for prothrombin time or partial thromboplastin time must not exceed 6 times the upper limit of the normal range.
- Laboratory tests from the screening visit will be used if the first [<sup>18</sup>F]FLT imaging session are performed within 28 days of the screening laboratory tests. Labs will be redrawn prior to [<sup>18</sup>F]FLT imaging session 1 if they are outside of the 28 day window prior to the scan.
- Within 96 hours prior to the second [<sup>18</sup>F]FLT imaging session the laboratory assessments will be performed. The values for this assessment must be within 4.0 X the upper or lower normal limits (unless clinically not relevant). For those patients receiving coumadin or another anticoagulant the upper limit for prothrombin time or partial thromboplastin time must not exceed 6 times the upper limit of the normal range.
- Within 96 hours prior to the possible third [<sup>18</sup>F]FLT imaging session the laboratory assessments will be performed. The values for this assessment must be within 4.0 X the upper or lower normal limits (unless clinically not relevant). For those patients receiving coumadin or another anticoagulant the upper limit for prothrombin time or partial thromboplastin time must not exceed 6 times the upper limit of the normal range.
- Includes: Heart Rate, Blood Pressure, and Temperature. Vitals signs will be collected prior to infusion and again at the completion of the imaging study. Height and weight will be recorded at the beginning of an imaging session.
- To be collected prior to the first imaging time point of each session, regardless of whether [<sup>18</sup>F]FLT or [<sup>18</sup>F]Fluciclovine is the first imaging to take place.
- For patients enrolling under Group B, laboratory tests from the screening visit will be used if they are performed within 28 days of the imaging session.

### **Multi-Tracer PET Imaging Visit:**

Imaging of the two PET tracers will be performed on separate days. The two days of imaging will be scheduled according to scanner and cyclotron availability, and all days of imaging will be obtained within one to two weeks of each other.

**APPENDIX C: [<sup>18</sup>F]FLT Infusion Flow Chart for Staff**

Subject Name and MRN: \_\_\_\_\_ Subject Study ID (if enrolled): \_\_\_\_\_

Projected Study Start Date: \_\_\_\_\_ Referring MD: \_\_\_\_\_

**[<sup>18</sup>F]Fluciclovine and [<sup>18</sup>F]FLT PET/CT Assessment of Primary High-Grade Brain Tumors**

IMAGING VISIT 1 (Baseline):  IMAGING VISIT 2 (Follow-up):

IMAGING VISIT 3 (Pseudoprogession):

The infusion and imaging procedure will be terminated in any patient who exhibits anaphylaxis, significant hypotension (systolic blood pressure less than 80 mmHg, dyspnea, chest pain, grand mal seizure or an O<sub>2</sub> saturation lower than 80%.

Administered dose of [<sup>18</sup>F]FLT \_\_\_\_\_ mCi

Specific Activity of [<sup>18</sup>F]FLT \_\_\_\_\_ Ci/mmol

Initials of Person Making Assessment	Time	Study	Temp	BP	HR	Weight	Height
		Baseline					
		End of Study				NA	NA

Completed by: \_\_\_\_\_

Date: \_\_\_\_\_

PI Signature: \_\_\_\_\_

Date: \_\_\_\_\_

**APPENDIX D: [<sup>18</sup>F]Fluciclovine Infusion Flow Chart for Staff**

Subject Name and MRN: \_\_\_\_\_ Subject Study ID (if enrolled): \_\_\_\_\_

Projected Study Start Date: \_\_\_\_\_ Referring MD: \_\_\_\_\_

**[<sup>18</sup>F]Fluciclovine and [<sup>18</sup>F]FLT PET/CT Assessment of Primary High-Grade Brain Tumors**

IMAGING VISIT 1 (baseline):  IMAGING VISIT 2 (follow-up):

IMAGING VISIT 3 (Pseudoprogession):

The infusion and imaging procedure will be terminated in any patient who exhibits anaphylaxis, significant hypotension (systolic blood pressure less than 80 mmHg, dyspnea, chest pain, grand mal seizure or an O<sub>2</sub> saturation lower than 80%.

Administered dose of [<sup>18</sup>F]Fluciclovine \_\_\_\_\_ mCi

Specific Activity of [<sup>18</sup>F]Fluciclovine \_\_\_\_\_ Ci/mmol

Initials of Person Making Assessment	Time	Study	Temp	BP	HR	Weight	Height
		Baseline					
		End of Study				NA	NA

Completed by: \_\_\_\_\_

Date: \_\_\_\_\_

PI Signature: \_\_\_\_\_

Date: \_\_\_\_\_

**APPENDIX E: [<sup>18</sup>F]FLT Patient Adverse Event Questionnaire**

Subject Name and MRN: \_\_\_\_\_ Subject Study ID (if enrolled): \_\_\_\_\_

Projected Study Start Date: \_\_\_\_\_ Referring MD: \_\_\_\_\_

IMAGING VISIT 1 (baseline):  IMAGING VISIT 2 (follow-up):  IMAGING VISIT 3 (Pseudoprogression):

POSSIBLE ADVERSE EVENT	Y =Yes N = No	Comment on possible AE
<b>Body as a Whole:</b>		
Pain (Abdominal)		
Pain (Chest/Breast)		
<b>PAIN (OTHER SITE)</b>		
Fever		
Injection site reaction		
<b>Cardiovascular System:</b>		
Vasodilation (flushing)		
<b>Digestive System:</b>		
Nausea		
Diarrhea		
Vomiting		
<b>Respiratory System:</b>		
Dyspnea (Shortness of breath)		
<b>Skin and Appendages:</b>		
Rash		
Pruritus (Itching)		
Urticaria (Hives)		
Sweating		
Cyanosis (discoloration of fingers/toes)		
<b>Central Nervous System</b>		
Visual disturbances		
Numbness of feet		
Numbness of fingers/hands		
Weakness of feet		
Weakness of fingers		
Burning sensation in feet		
Burning sensation of fingers		

Performed by: \_\_\_\_\_

Date: \_\_\_\_\_

PI Signature: \_\_\_\_\_

Date: \_\_\_\_\_

### APPENDIX F: [<sup>18</sup>F]Fluciclovine Patient Adverse Event Questionnaire

Subject Name and MRN: \_\_\_\_\_ Subject Study ID (if enrolled): \_\_\_\_\_

Projected Study Start Date: \_\_\_\_\_ Referring MD: \_\_\_\_\_

IMAGING VISIT 1 (baseline):  IMAGING VISIT 2 (follow-up):  IMAGING VISIT 3 (Pseudoproggression):

POSSIBLE ADVERSE EVENT	Y =Yes N = No	Comment on possible AE
<b>Body as a Whole:</b>		
Pain (Abdominal)		
Pain (Chest/Breast)		
<b>PAIN (OTHER SITE)</b>		
Fever		
Injection site reaction		
<b>Cardiovascular System:</b>		
Vasodilation (flushing)		
<b>Digestive System:</b>		
Nausea		
Diarrhea		
Vomiting		
<b>Respiratory System:</b>		
Dyspnea (Shortness of breath)		
<b>Skin and Appendages:</b>		
Rash		
Pruritus (Itching)		
Urticaria (Hives)		
Sweating		
Cyanosis (discoloration of fingers/toes)		
<b>Central Nervous System</b>		
Visual disturbances		
Numbness of feet		
Numbness of fingers/hands		
Weakness of feet		
Weakness of fingers		
Burning sensation in feet		
Burning sensation of fingers		

Performed by: \_\_\_\_\_

Date: \_\_\_\_\_

PI Signature: \_\_\_\_\_

Date: \_\_\_\_\_

**APPENDIX G: NCI Common Toxicity Criteria**

[https://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/docs/CTCAE\\_v5\\_Quick\\_Reference\\_5x7.pdf](https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf)