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**TITLE: Cellular Proliferation Imaging Using [¹⁸F] fluorothymidine (FLT)
Positron Emission Tomography (PET) in Brain Tumors**

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6.iii.1. SCHEMA

This clinical study will obtain preliminary data to investigate the role of [¹⁸F]FLT in patients with primary or recurrent brain tumors that will be undergoing tumor therapy. This study will obtain data on the value of [¹⁸F]FLT to quantify uptake changes related to cellular proliferation using serial scans, which will include a baseline scan prior to therapy, and follow-up scans after standard therapy has started. If promising, these data will be used to design larger clinical trials.

Cellular proliferation imaging with specific PET agents provides a unique approach to quantify the growth rate of tumors and assess their treatment response. Our research group pioneered the development and validation of [¹⁸F]fluorothymidine (FLT) as a tracer of cellular proliferation. FLT has been shown to accumulate in tissues in proportion to the level of *thymidine kinase 1* activity. This enzyme is up-regulated during the DNA synthesis portion of the cell cycle and therefore its activity reflects cellular proliferation. We will use this FLT biochemical marker using PET/CT to quantify regional cellular growth rate in brain tumors. These tumors are known to have high levels of proliferation, which can change with treatment. Our hypothesis is that quantifying cellular proliferation in brain tumors will provide a useful measure of disease activity and location.

STUDY PROTOCOL SEQUENCE

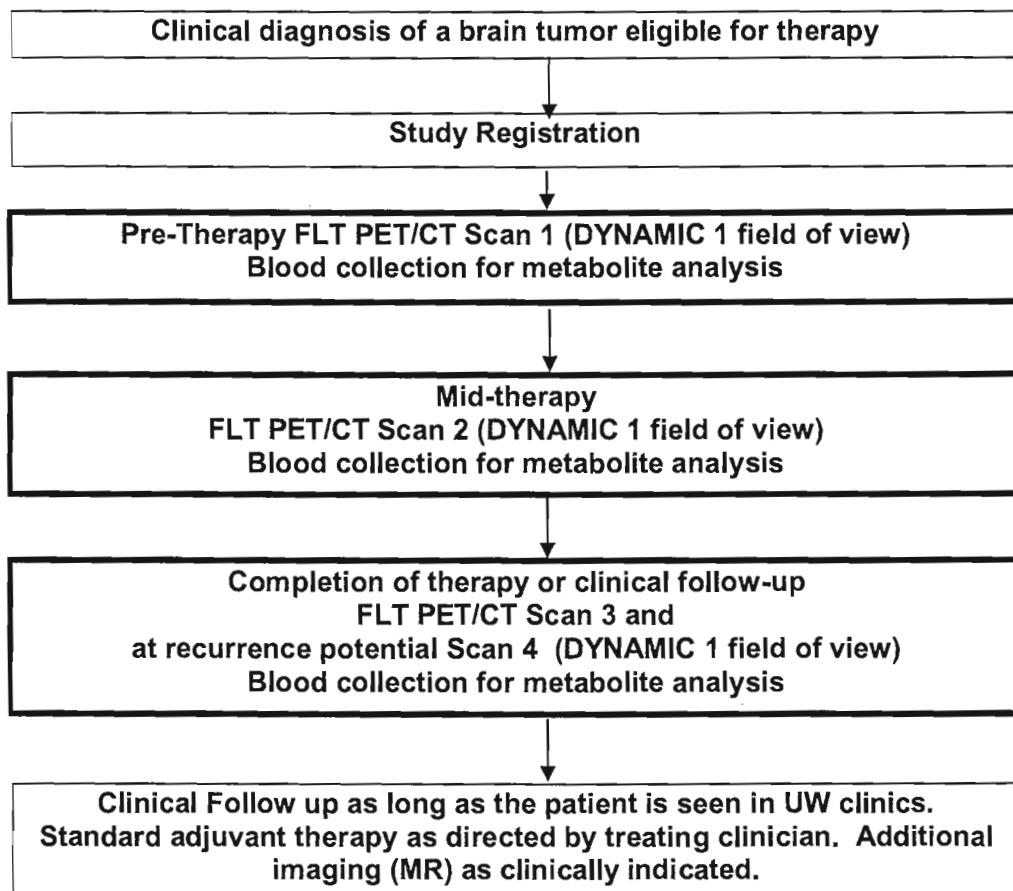


Figure 6.iii.1. Schema for brain tumor protocol

We emphasize that this is an observational study in that [¹⁸F]FLT PET/CT will not be used to direct therapy. Treatment regimens are chosen by the referring oncologist prior to the [¹⁸F]FLT PET study on the basis of clinical criteria. While patients and referring physicians will not be blinded to [¹⁸F]FLT PET results, treatment choices will be made prior to the entry of the patient into the study and clinical management will be based on usual standard-of-care anatomic MRI and/or FDG PET/CT and will not be altered because of the FLT PET/CT results.

6.iii.2. OBJECTIVES

6.iii.2.a. Specific Aims:

- 6.iii.2.a.1.** Using FLT PET/CT as a measure of cellular proliferation, assess tissue proliferation in disease sites of brain tumor patients before therapy (surgery, chemotherapy or radiotherapy or any combination of these.).
- 6.iii.2.a.2.** Determine level of change in cellular proliferation compared with baseline (Scan 1) in brain tumors at mid-therapy (Scan 2), after completion of therapy (Scan 3) and in the clinical follow-up period (Scan 4), when possible.
- 6.iii.2.a.3.** Correlate levels of cellular proliferation measured by FLT PET/CT at baseline and treatment-induced changes in brain tumor proliferation with clinical response status (clinical categories are complete remission, lesser degrees of response/stable disease, and no response).
- 6.iii.2.a.4.** Assess spatial heterogeneity of FLT uptake to identify local differences in brain tumor disease burden. This aim is a secondary analysis. For patients in which re-operation is clinically indicated, we will mark surgical specimens to co-register with regions of the FLT image. These tissues will be banked for future histological assays.

6.iii.3. BACKGROUND

Cellular proliferation imaging with specific PET agents provides a noninvasive approach to quantify the growth rate of tumors serially and to assess their treatment response. Our research group pioneered the development and validation of [¹⁸F]-fluorothymidine (FLT), which has been shown to accumulate in tissues in proportion to the level of cellular growth (Shields, 1998; Krohn, 2012). During cellular uptake, FLT is transported across the cell membrane and then phosphorylated by *thymidine kinase-1*, resulting in intracellular trapping of the radiolabeled nucleotide (Grierson, 2004). Because of the substitution at the 3'-position, FLT is not a good substrate for *DNA polymerase*. *Thymidine kinase-1* activity is controlled throughout the cell cycle processes, which forms the basis for relating FLT uptake to cellular proliferation. Although FLT is not incorporated into DNA, its retention in cells is directly related to *thymidine kinase-1* activity, which peaks in S-phase and then is destroyed as the cells proceed to division (Weber, 1991; Eriksson, 1994). Our research has shown that uptake is also influenced by blood flow, which led us to develop a simple dynamic PET imaging method to obtain quantitative tissue proliferation rate information separate from tumor blood flow (Muzi

2006, Wells 2002a). PET has the ability to image the whole body and yield quantitative regional tissue information on a specific biological process. We will exploit this approach in this study of FLT PET/CT to quantify regional cellular growth rate in primary brain tumors.

6.iii.3.a. Study Disease

Brain tumors: Brain tumors are known to have variable levels of cellular proliferation which is also thought to be heterogeneous in the tumor volume. These tumor characteristics may be very important factors in treatment resistance. Our hypothesis is that quantifying cellular proliferation in brain tumors will provide a useful measure of disease activity. The brain malignancies are a group of tumors where dramatic responses with long-term survival can occur, but also where many patients' exhibit treatment resistance and have very short survival.

Standard initial treatment for glioblastoma currently consists of surgical removal of as much of the MRI contrast-enhancing lesion as can be accomplished safely, followed by radiotherapy and temozolomide (TMZ) chemotherapy for 6 weeks. Unfortunately, early treatment failures occur in 30-50% of patients. This definition of treatment failure is described as an increase in the MRI T1+Gd tumor volume (de Wit, 2004). Immediate post-radiotherapy MRI changes in malignant glioma can mimic tumor progression (Taal, 2008). The incidence of this pseudo-progression in a cohort of malignant glioma patients treated with chemo-radiation with temozolomide is significant (Brandes, 2008). From 28-52% of these patients will demonstrate improvement on subsequent MRI studies when they continue on standard TMZ chemotherapy. This situation defines pseudo-progression as opposed to true disease progression, where continued treatment with temozolomide is associated with continued clinical decline and MRI T1+Gd worsening. Clinicians armed with anatomic MRI imaging alone may wrongly conclude that standard treatment is failing in these patients, leading to cessation of a treatment that is actually working. Also, misdiagnosing pseudo-progression in patients who are responding could risk entering such patients into phase II or III trials of new agents, decreasing the statistical power of the study and potentially leading to falsely positive outcomes. On the other hand, continuing TMZ in the patients that are true progressors wastes their time and physiological resources when they should be offered second line treatment or enrollment in a phase II or III research treatment protocol. Thus, the ability to make the important distinction between progression and pseudo-progression is critical for the care of treated brain tumor patients and could potentially change patient management. In some patients, the neuropathologist who reviews the resected tumor resolves the question of pseudo-progression versus true progression. Ideally, however, noninvasive imaging methods should be developed to answer this question reliably. Our primary hypothesis is that FLT PET/CT should be able to distinguish between when the patient has true tumor progression and needs to change therapy and when the MRI contrast is reflecting pseudo-progression and the patient should continue with TMZ chemotherapy. That way, the 50% or possibly more true progressors could avoid continuing futile TMZ.

Recent reviews have described many alternative strategies for overcoming treatment resistance for therapy with new combination protocols. One example is the addition of bevacizumab to the TMZ-radiotherapy combination to normalize vascular permeability changes at the time of radiologic signs of pseudo-progression, but the change in patient outcome for this therapy addition is unknown (Villano, 2009). Increases in radiotherapy

with stereotactic and brachytherapy techniques are also showing results with cautious increases in treatment dose, and little increased toxicity (Fokas, 2009, Fabrini, 2009). Other attempts at tumor re-resection in patients with recurrent tumors or progression is historically shown to be effective for only a subset of patients, leading to exploration of the clinical utility of drugs designed to target specific tumor mechanisms for cell growth and maintenance. However, many of these targeted agents have not demonstrated improved survival in groups of non-selected patients (Sathornsumetee, 2009). While MR methods in aggregate may have the ability to distinguish true treatment responses prospectively, there is little agreement on techniques to apply consistently to make this critical clinical diagnosis in an individual patient. The new research of this study will evaluate PET approaches to these important clinical questions. If FLT PET/CT can accurately identify true progression, then this imaging protocol can be used for following response to current therapy and for objective evaluation of the promise of newer molecularly targeted therapies for patients with malignant brain tumors. Tumor proliferation imaging will enable us to observe the location and distribution of disease throughout the course of treatment to gain new insight into a patient's progress and about brain tumor biology. The goal of this project is to provide objective and quantitative imaging information about the entire brain tumor in a pilot study that builds on our substantial work in FLT-PET applied to human cancer.

6.iii.3.b. Investigational Agent

3'-Deoxy-3'-[¹⁸F]fluorothymidine ([¹⁸F]FLT) is a structural analog of the DNA constituent, thymidine (Figure 6.iii.2). It is a radiolabeled imaging agent that has been proposed for investigating cellular proliferation with positron emission tomography (PET). Although [¹⁸F]FLT is not incorporated into DNA, it is trapped in the cell due to phosphorylation by *thymidine kinase 1*, a part of the salvage pathway. As such, it has potential as a marker of proliferating tumor in proportion to the DNA synthesis rate. Therefore, [¹⁸F]FLT is proposed as a radiolabeled imaging probe for *in vivo* assessment of cellular proliferation in malignant tumors using PET. For complete information, please refer to the Investigator's Brochure: "3'-deoxy-3'-fluorothymidine: [¹⁸F]FLT, An Investigational Positron Emission Tomography (PET) Radiopharmaceutical for Injection and intended for use as an *in vivo* diagnostic for imaging active cellular proliferation of malignant tumors."

6.iii.3.c. Rationale

[¹⁸F]FLT is a radiolabeled imaging probe for *in vivo* assessment of cellular proliferation using PET. Preclinical and clinical studies suggest that early imaging of response using this radiopharmaceutical can demonstrate effectiveness of treatment in a variety of tumors. In this protocol, we will assess regional FLT uptake in patients with malignant brain tumors.

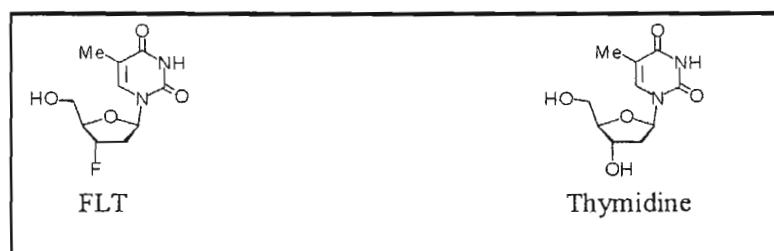


Figure 6.iii.2. Molecular structures for FLT and thymidine

Specifically, we propose to use [¹⁸F]FLT PET/CT to assess changes in imaging measures in the clinical course of brain tumor patients. Subsequent studies (not part of this trial) could eventually allow treatments to be tailored to the tumor response measured by imaging, eliminating the use, costs and toxicity of ineffective treatments and improving the patient's quality of life. This is our long-term objective. In this protocol, FLT PET/CT imaging measures will be compared with clinical measures of tumor response to test specific hypotheses.

Hypotheses for this protocol.

1. Quantifying tumor proliferation in brain tumors will provide a reliable measure of disease. For example, kinetic analysis of the FLT-PET/CT data will allow distinction between true progression and pseudo-progression in patients with glioblastoma who have finished surgery plus radiotherapy and temozolomide and whose measurable disease by standard MRI after initial treatment has appeared to progress.
2. Changes in tumor proliferation (FLT flux) as determined by kinetic analysis of FLT-PET/CT imaging will correlate with clinical treatment response.
3. Brain tumor cellular proliferation is heterogeneous. This heterogeneity can be quantified by the FLT PET/CT procedure and is an independent predictor of response.

6.iii.4. PARTICIPANT SELECTION

6.iii.4.a. Inclusion Criteria:

- 6.iii.4.a.1. At least 18 years of age.
- 6.iii.4.a.2. Have a diagnosis or suspected diagnoses of a brain tumor (primary, recurrent, or metastatic) by standard clinical diagnosis such as pathology or imaging.
- 6.iii.4.a.3. Planned for treatment with radiation, chemotherapy and surgical resection or any of these treatment strategies combined. The clinical treatment will not be influenced by the experimental imaging studies described in this protocol.

6.iii.4.b. Exclusion Criteria:

- 6.iii.4.b.1. Inability to provide informed consent
- 6.iii.4.b.2. Pregnancy
- 6.iii.4.b.3. Inability to lie still for the imaging study
- 6.iii.4.b.4. Weight over 350 lbs., the weight limit of the tomograph table.

6.iii.4.c. Inclusion of Women and Minorities

Patients 18 years of age or older with a brain tumor and planning to undergo therapy will be eligible for study participation.

No other discriminatory factors, including age, sex, or ethnic background will be used to determine eligibility. Every effort will be made to ensure that minorities are recruited for study participation.

6.iii.4.d. Accrual Targets

Brain tumors in adults may have low incidence in some ethnic and racial groups. We have not excluded any ethnic or racial groups from participation in our studies and will continue this policy, but anticipate that some ethnic and racial groups will not enroll due to the rarity of presentation. (Table 6.iii.1) The targeted enrollment is for a total of 30 patients with three to four FLT PET/CT scans per patient. We recognize that some patients, though willing, will be too sick to be able to complete three studies which is the number required for the power calculations described in Data Analysis, section 6.iii.14.b. From our experience with this patient group, we estimate that 25% of our patients will only receive one or two scans. Therefore, we will enroll up to 40 subjects to the study. The current funding level of our NCI Program Project imposes a practical limit on the total accrual number. We fully appreciate that this will impact the statistical power. Nevertheless, because proliferation changes greatly with therapy, we are optimistic that the study will lead to significant findings on our proposed hypotheses (see Statistical Analysis, section 6.iii.14).

Table 6.iii.1. Targeted Enrollment.

TARGETED/PLANNED ENROLLMENT: Number of Subjects (<i>must provide actual numbers. (i.e. no range)</i>)			
Ethnic Category	Sex/Gender		
	Females	Males	Total
Hispanic or Latino	2	3	5
Not Hispanic or Latino	14	21	35
Ethnic Category Total of All Subjects*	16	24	40
Racial Categories			
American Indian/Alaska Native	1	2	3
Asian	0	1	1
Native Hawaiian or Other Pacific Islander	0	1	1
Black or African American	0	1	1
White	15	19	34
Racial Categories: Total of All Subjects	16	24	40

6.iii.5. REGISTRATION PROCEDURES

Not applicable; this is a single institution trial.

6.iii.6. TREATMENT PLAN

This is not a treatment trial. Patients will be managed according to the plan developed by their treating clinicians. Patients will be treated at the University of Washington clinics where standard of care for treatment calls for surgery, chemotherapy, radiation, or chemoradiation with adjuvant chemotherapy. Standard salvage therapy will be offered patients who progress based on current clinical indications by their clinicians. Salvage therapy includes re-operation, re-irradiation, second line chemotherapy agents or a combination of these options. We will serially image patients using FLT PET/CT for quantifying tumor cellular proliferation at several time points in their clinical course. The times will include baseline and can include mid therapy and up to two scans during the clinical follow-up period after therapy. Standard clinical MR images will be acquired during the therapy schedules as requested by the clinicians. Patients may also undergo research MR imaging.

6.iii.6.a. [¹⁸F]FLT Administration:

[¹⁸F]FLT will be administered in the PET Imaging Suite at the University of Washington Medical Center or at the Seattle Cancer Care Alliance (SCCA). Upon arrival, the patient will have an opportunity to have questions answered regarding the procedure. The patient will have one intravenous line placed prior to [¹⁸F]FLT administration. The patient will then be positioned in the PET scanner followed by a transmission scan using a low-dose CT scan. Then [¹⁸F]FLT will be administered intravenously by a physician. The administered dose will be 0.07 mCi/kg with a maximum of 5 mCi. The drug solution is stored at room temperature in a gray butyl septum sealed, sterile, pyrogen-free glass vial and has an expiration time of 8 hours. The injectable dose of [¹⁸F]FLT will be \leq 0.07 mCi/kg of fluorine-18, not to exceed 5 mCi. In the dose of [¹⁸F]FLT, only a small fraction of the FLT molecules are radioactive. The amount of injected drug is \leq 6.1 μ g (\leq 25 nmol per dose) of FLT. There is no evidence that nonradioactive and radioactive FLT molecules display different biochemical behavior. [¹⁸F]FLT is administered to subjects by intravenous injection of \leq 10 mL. The injection will be infused over approximately 1 minute and followed by a saline flush. The infusion and imaging procedure will be terminated in any patient who exhibits anaphylaxis, significant dyspnea or chest pain.

The adverse events to be specifically monitored during the infusion include localized discomfort at the IV injection site, pain, respiratory difficulties, flushing, dizziness, pruritus/rash, and any other symptoms that could be secondary to an anaphylactic reaction. The subject will be instructed to report any subjective symptoms or sensory changes noted. All adverse events occurring post [¹⁸F]FLT infusion will be recorded within a 24-hour period.

6.iii.6.b. Study Procedures and Schedule of Events:

6.iii.6.b.1. Initial visits prior to [¹⁸F]FLT PET: Patients who are identified as potential candidates for the [¹⁸F]FLT PET study will be approached to explain the nature of the study and to obtain their written informed consent to be enrolled in the protocol. The following additional patient data will be obtained: tumor location, correlative imaging findings, histologic diagnosis (if available), age at diagnosis, and gender.

6.iii.6.b.2. Day of [¹⁸F]FLT PET Scans: The patient will be positioned supine in the PET/CT scanner for a dynamic scan over the brain. [¹⁸F]FLT PET/CT will be performed using the standard dose of 5 mCi. Patients will have 1 or 2 intravenous lines placed. A low-dose CT scan (PET/CT) will be used for positioning and attenuation correction. The single field of view over the brain will be imaged dynamically for about 90 minutes following the start of a one-minute intravenous infusion of [¹⁸F]FLT. Total patient time, including line placement and imaging is typically 2 hours. During the scan, three venous blood samples will be taken to analyze for FLT metabolites by the method we developed and is detailed in the Investigator's Brochure our previously developed solid phase exchange method that is published on the ACRIN trials website (ref: <http://www.acrin.org/Portals/0/Protocols/6689/imaging/ACRIN%206689%20FLT%20Blood%20Processing%20-20V1.0%20%20%20%20February%202011,%202011.pdf>).

6.iii.6.b.3. After the baseline and follow up [¹⁸F]FLT PET Scans: A follow up phone call will be made by study staff the next day after a [¹⁸F]FLT PET/CT scan to ask about any signs of adverse effects.

6.iii.6.c. General Concomitant Medication and Supportive Care Guidelines

There are no requirements for concomitant medication supportive care as part of this study.

6.iii.6.d. Duration of Therapy:

As this is an observational study, the results of [¹⁸F]FLT PET/CT are not used to direct therapy. The duration of therapy is determined on the basis of clinical grounds by the referring physician, and is not influenced by the FLT study.

6.iii.6.e. Duration of Follow Up:

Patients will be followed until the time of their treatment failure or they are otherwise lost to follow-up, at which time they are considered off study. This is typically 5 years or less but may be as great as 7 years. Patients lost to follow-up will be tracked for overall survival to every extent possible based on information from public databases.

6.iii.7. EXPECTED TOXICITIES AND DOSING DELAYS / DOSE MODIFICATIONS

Not applicable to an observational imaging trial.

6.iii.8. ADVERSE EVENTS REPORTING

Qualifying Adverse Events (AEs), including Serious Adverse Events (SAEs), as defined herein, will be reported via the FDA Adverse Event Expedited Reporting System (AERS). For this IND we will report adverse events based on the FDA final rule for IND safety reporting requirements under 21 CFR part 312 published on September 29, 2010 and implemented on March 28, 2011. This investigational study is not a BA or BE study so 21 CFR part 320 is not applicable. Adverse events will also be reported to the local IRB according to their requirements.

6.iii.8.a. General Definitions (from 21 CFR 312.32 (a))

Adverse Event (AE): An **Adverse Event** is an untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related. For this study the drug is [¹⁸F]FLT and adverse events would include any events experienced by a study participant **during the Adverse Event reporting period defined in Table 6.iii.4 Study Calendar** whether or not it was considered to be related to the [¹⁸F]FLT.

Adverse Reaction: An **Adverse Reaction** is any adverse event caused by a drug. In this study the drug is [¹⁸F]FLT.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

An **adverse event or suspected adverse reaction** is considered "**unexpected**" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

An **adverse event or suspected adverse reaction is considered "serious"** if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events

include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

An **adverse event or suspected adverse reaction is considered “life-threatening”** if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Investigational Agent: An investigational agent is any agent held under an Investigational New Drug (IND) application. For purposes of this study, 3'-deoxy-3'-[¹⁸F]-fluorothymidine ([¹⁸F]FLT) is the investigational agent.

6.iii.8.b. AE Reporting Requirements

The investigators on this protocol will report any suspected adverse events that occur after [¹⁸F]FLT administration and within the specified follow-up period to the sponsor, J. Link, and they will work together to determine whether there was an adverse event or adverse reaction and the severity of the adverse event or reaction.

All AEs will be followed by the investigators until resolution, stabilization, scientifically and clinically satisfactory explanation as to attribution and etiology is achieved, or until subject is lost to follow up.

6.iii.8.c. CAEPR / ASAE for 3'-deoxy-3'-[¹⁸F]fluorothymidine. (NSC 743144)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. The Agent Specific Adverse Event List (ASAE) would include the expected adverse events associated with the use of [¹⁸F]FLT. At this time there have been no reported AEs associated with the use of [¹⁸F]FLT and so no ASAE are expected as a result of IV injection of [¹⁸F]FLT in our study population with the specifications for our drug product (Section 7 of this IND). We will continue to update our CAEPR and ASAE lists as this study progresses, including by reviewing the literature and our in-house data safety monitoring. If any are found we will begin a ASAE list. Any information on reported AEs for [¹⁸F]FLT will be provided by the sponsor to all of the investigators on this protocol.

6.iii.8.d. Potential but Unexpected Adverse Events for [¹⁸F]FLT

The following are extremely rare potential risks that are associated with the use of IV injected radiopharmaceuticals that could apply to the IV administration of [¹⁸F]FLT.

There is the potential with intravenous injections, including [¹⁸F]FLT, for allergic reactions and potentially anaphylaxis. This would be a serious to life threatening adverse event. This has not been observed in limited human

exposure to date. Throughout the PET procedure, patients will be monitored by trained personnel for any symptoms or signs of an allergic reaction. Emergency equipment and medical personnel are available in the event of an allergic reaction.

The injection site may become infected.

The dose might be extravasated leading to localized pain / discomfort.

3'-Deoxy-3'-[¹⁸F]fluorothymidine is a positron emitting radiopharmaceutical. As such, it poses an intrinsic radiation exposure risk. However, when administered in accordance with the Investigator's Brochure as a PET imaging agent, this risk is felt to be extremely small. The organ and total body doses associated with FLT PET imaging are comparable to those associated with other widely used clinical nuclear medicine procedures and are presented in Section 10 of this IND application.

Note: Although not previously reported, in combination with other agents, it is possible that [¹⁸F]FLT could exacerbate an adverse event currently known to be caused by an other agent, or might result in adverse events that have not previously been associated with either agent.

6.iii.8.e. Review of Safety Information.

As required by 21 CFR 312.32(b), the sponsor will promptly review all information relevant to the safety of the drug. This work will in all cases be done in close collaboration with the physician investigators for this protocol. The physician investigators will be providing much of this information to the local IRB as well for data safety and review monitoring. The sponsor's review will include determining whether there is a safety event over time and the causality. As there are no expected events all events that will be monitored will be unexpected. Reporting will be as described in Table 6.iii.1.

Characterization of the severity of an Adverse Event: Adverse events will be graded as below and based on the definitions in section 6.iii.8.1.

Grade: Grade denotes the severity of the AE. An AE is graded using the following categories:

- **Mild**
- **Moderate**
- **Severe**
- **Life-threatening or disabling**
- **Fatal**

NOTE: Severity is graded on the Cancer Therapy Evaluation Program (CTEP) Common Terminology Criteria for Adverse Events (CTCAE) based scale for each adverse event. For example, an abnormal hemoglobin value is graded for severity from 1 to 5 [death] based upon where that value falls on the CTCAE scale of abnormal hemoglobin values. "Severity" is NOT the same as

"Seriousness." All appropriate clinical areas should have access to a copy of the most current CTCAE and a copy of the CTCAE can be downloaded from (<http://ctep.cancer.gov>).

Attribution of cause: The physician investigators will determine whether an adverse event was related to a medical treatment or procedure. Definitions taken from our work with CTEP and NIH give the following definitions for "Attribution" that we will adopt for this IND study: Attribution is a clinical determination, by the investigator, as to whether an AE is related to a medical treatment or procedure. Attribution categories are:

- **Definite:** The AE is **clearly related** to a treatment or procedure
- **Probable:** The AE is **likely related** to a treatment or procedure
- **Possible:** The AE **may be related** to a treatment or procedure
- **Unlikely:** The AE is **likely unrelated** to a treatment or procedure
- **Unrelated:** The AE is **clearly not related** to a treatment or procedure

NOTE: Attribution is part of the assessment of an adverse event. Determining that an event is 'unlikely related' or 'unrelated' to a study agent or procedure does NOT make the event unreportable, or disqualify the event as an AE. As defined above, an AE is reportable as specified herein if it occurred: "**during the Adverse Event reporting period defined in the protocol**, or by applicable guidance, regulation, or policy."

6.iii.8.f. Adverse Event Reporting

Expedited AE reporting for this study will be done through the Cancer Consortium IRB and FDA and as required by FDA MedWatch. These requirements are briefly outlined in the table below.

Table 6.iii.2 Reporting Requirements

	Unexpected		Expected
	Adverse Reaction (known or suspected attributable to the use of [¹⁸ F]FLT)	AE not attributable to [¹⁸ F]FLT	AE, AR
	Serious including life-threatening (or death)	Nonserious	Life-Threatening or serious or not serious
Reporting Time Requirement to the FDA	Report to FDA ASAP and within 7 days of discovery of event (section 6.iii.8.7)	Annual Continuation Review submission	Annual Continuation Review submission
Reporting Form for the FDA	IND Safety report of potentially serious risk	Annual Reports / Case reports	Not applicable to [¹⁸ F]FLT
Reporting Time Requirement to the local IRB	Report to IRB ASAP within 10 days of discovery of event (suspected is defined as 50% probability attributable to [¹⁸ F]FLT study) this also includes any increased risks with the study even without an AE	At continuation review time	At continuation review time
Reporting form for the IRB	Expedited Reporting Form for Unanticipated Problems or Noncompliance and Adverse Event Reporting Form	Form for Unanticipated Problems or Noncompliance, Case reports on continuation form, Data Safety Monitoring Reports	Form for Unanticipated Problems or Noncompliance, Case reports on continuation form, Data Safety Monitoring Reports

6.iii.8.g. Expedited Adverse Reaction Reporting Guidelines

Life-threatening (or fatal) adverse reactions must be reported within 7 days to the FDA. The FDA should be notified as soon as the adverse reaction is discovered by telephone or fax or email. The instructions and forms are available at <http://www.fda.gov/Safety/MedWatch/HowToReport/default.htm>. The report should be sent ASAP by mail and followed with a follow-up report. Individual IND safety reports to FDA are submitted on the Medwatch FDA Form 3500A as an "IND Safety Report". The form should be sent to The Director, Office of Generic Drugs in the Center for Drug Evaluation and Research at FDA. The address and phone numbers are available at: <http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm119100.htm>.

All life threatening adverse reactions reports are submitted to the FDA, our local IRB and to all investigators. A copy of the report is kept on file.

6.iii.8.h. Protocol-Specific Expedited Adverse Event Reporting Exclusion

Not applicable for this IND agent

6.iii.8.i. Routine Adverse Event Reporting

Routine Adverse Event Reporting will be done to both the FDA and local IRB as detailed in the table 6.iii.2 .

6.iii.9. PHARMACEUTICAL INFORMATION

6.iii.9.a. Production of the Radiopharmaceutical

3'-Deoxy-3'-[¹⁸F]fluorothymidine (FLT): will be produced at the University of Washington according to the methods described in Section 7 of this IND (Chemistry, Manufacturing and Controls).

6.iii.9.b. Reported Adverse Events and Potential Risks

A list of the adverse events and potential risks associated with [¹⁸F]FLT are provided in Section 6.iii.8.

6.iii.9.c. Agent Accountability

The chemical precursor for [¹⁸F]FLT is provided by ABX in single-use, 10 mg vials that are delivered to the custody of Dr. Steven Shoner, the lead radiochemist for this project. They are stored in a controlled temperature refrigerator in a locked and secure room and they are inventoried with a chain of custody maintained from the time of receipt. Each radiosynthesis is done by Dr. Shoner, Dr. Krohn or their designee and, after passing all required quality control assays, the product [¹⁸F]FLT dose is drawn up under the authority of the nuclear medicine physician co-investigator, Dr. Andrew Shields, Dr. Jean Lee or their designee. The quality control tests that must be passed prior to release of the product [¹⁸F]FLT for injection include the pH, radioactive purity, radiochemical purity, specific activity, sterilizing filter integrity, tests for Kryptofix [2.2.2], acetonitrile, acetone, dimethylsulfoxide, endotoxins and particulates. Acceptance criteria are detailed in the CMC section of this IND. The [¹⁸F]FLT dose is drawn up into a syringe, assayed for mCi at time of injection, labeled and passed to the physician investigator who oversees the re-assay of the dose and the administration to the research subject.

6.iii.10. CORRELATIVE / SPECIAL STUDIES

Study data are comprised of imaging parameters from at least two and as many as four [¹⁸F]FLT PET studies.

6.iii.10.a. [¹⁸F]FLT PET/CT Image Analysis

Preliminary data suggests that [¹⁸F]FLT is essentially irreversibly phosphorylated and trapped inside cells during the imaging period. Prior to this point, however, there is a flux of unphosphorylated [¹⁸F]FLT (reversible component). If the rate of phosphorylation (k_3) is low, then the early contribution of perfusion to the measured tumor activity is high. Assuming the phosphorylation rate correlates to the proliferative rate, a high rate of phosphorylation means that the cumulative measured tumor activity should be representative of cellular proliferation.

Volumes of interest (VOIs) will be drawn around the target lesions on the [¹⁸F]FLT images based on the tumor localization on the baseline CT. [¹⁸F]FLT PET studies will be registered based on their corresponding CT transmission scans images, and time activity curves (TACs) will be calculated.

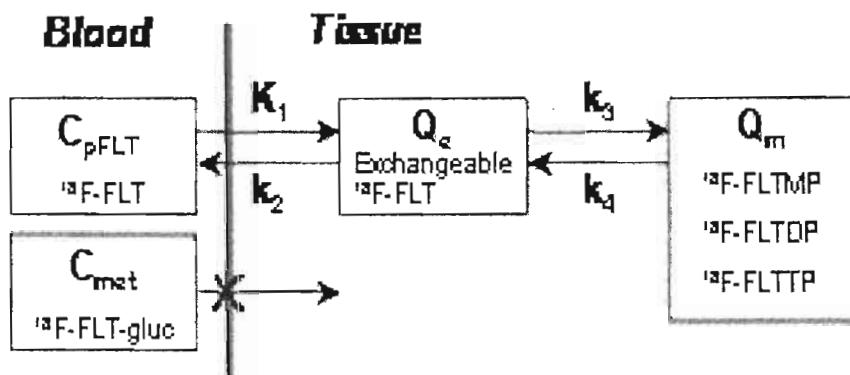


Figure 6.iii.3 Kinetic model of FLT metabolism is comprised of an exchangeable tissue compartment and a compartment of trapped FLT \ nucleotides. Four rate constants describe kinetic transfer rates between the 2 compartments. FLTMP = FLT-monophosphate; FLTDP = FLT-diphosphate; FLTTP = FLT-triphosphate; FLT-gluc = FLT-glucuronide; Q_e = exchangeable tissue compartment; Q_m = compartment of trapped FLT phosphorylated nucleotides; $C_{p\text{FLT}}$ = concentration of FLT in arterial plasma; C_{met} = concentration of metabolites in arterial plasma. An imaged extraction procedure will be used to recover the arterial input function (O'Sullivan, et al. 2010).

6.iii.10.a.1. Compartmental Analysis

From PET/CT VOI analysis of dynamic data, SUV (30-60 min summed) and parameters reflecting transport (K_1) and FLT flux (K_{FLT}) will be calculated using mathematical modeling analysis as described by Muzi et al (Muzi, 2005a; Muzi, 2005b). The change in these rate parameters between baseline and follow up imaging will also be calculated.

6.iii.10.b. Biologic Correlates

Blood Collection Time Points

Blood samples will be obtained from each participant at the time of dynamic PET imaging. Each blood sample is used both for the determination of radioactivity and metabolite analysis. Blood samples are drawn from patients using a second venous catheter placed in the opposite arm from the injection site or from a clinically placed PICC line or Port-a-cath. Blood samples of 2-3 mL each are collected into heparinized syringes (or a heparinized blood vacutainer) at 15, 30 and 60 minutes after ¹⁸F-FLT injection, which is infused over one minute.

No tissue sampling other than what is required for clinical diagnosis will be done for this study. At the time of resection (or re-resection), tissue not needed for standard clinical care may be labeled with anatomic location co-registered to the PET images and banked for potential future analysis of biomarkers. Any biomarker studies would be described in a separate IRB protocol and would be consented separately from the FLT PET/CT imaging protocol. This consent is already part of the consent for surgery.

6.iii.11. STUDY CALENDAR

Table 6.iii.4

	Pre-Study	Day of [¹⁸ F]FLT baseline	Mid-Rx	Post-therapy Day of [¹⁸ F]FLT (optional)	Post-Rx 1 year or time of suspected recurrence [¹⁸ F]FLT
Confirmed diagnosis of malignant brain tumor	X				
Informed Consent	X				
Demographics	X				
Pregnancy Test **		X		X	X
Vital Signs *	X				
[¹⁸ F]FLT Injection and Blood Collection		X	X	X	X
Adverse Event Evaluation		X (0-24hr)	X (0-24hr)	X (0-24hr)	X (0-24hr)

*Vital signs include heart rate and blood pressure. ** Females of childbearing potential will have a serum pregnancy test just prior to the first PET scan

6.iii.12. MEASUREMENT OF EFFECT

This protocol is limited to developing relationships between parameters determined from imaging studies and clinical outcome, which includes treatment assessment using clinical MRI scans. The definition of response is defined below using Tables 6.iii.5 and 6.iii.6 from Wen et al., (Wen 2010). The RANO criteria do not rely on two

dimensional volume assessments in the MRI image for determination of response status.

Table 6.iii.5. Criteria for Response Assessment Incorporating MRI and Clinical Factors (Wen 2010)

Response	Criteria
Complete response	Requires all of the following: complete disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks; no new lesions; stable or improved nonenhancing [T2/FLAIR] lesions; patients must be off corticosteroids [or on physiologic replacement doses only], and stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a complete response; the best response possible is stable disease.
Partial response	Requires all of the following: >50% decrease compared with baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks; no progression or nonmeasurable disease; no new lesions; stable or improved nonenhancing [T2/FLAIR] lesions on same or lower dose of corticosteroids compared with baseline scan; the corticosteroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan, and stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a partial response; the best response possible is stable disease.
Stable disease	Requires all of the following: does not qualify for complete response, partial response, or progression; stable nonenhancing [T2/FLAIR] lesions on same or lower dose of corticosteroids compared with baseline scan. In the event that the corticosteroid doses was increased for new symptoms and signs without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that the increase in corticosteroids was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.
Progression	Defined by any of the following: $\geq 25\%$ increase in sum of the products of perpendicular diameters of enhancing lesions compared with the smallest tumor measurement obtained either at baseline (or no decrease) or best response, one stable or increasing doses of corticosteroids*; significant increase in T2/FLAIR nonenhancing lesion on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy* not caused by comorbid events (e.g. radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects); any new lesion; clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication adverse effects, complications of therapy, cerebrovascular events, infection, and so on) or changes in corticosteroid dose; failure to return for evaluation as a result of death or deteriorating condition; or clear progression of nonmeasurable disease.
<p>NOTE: All measurable and nonmeasurable lesions must be assessed using the same technique as at baseline.</p> <p>Abbreviations: MRI, magnetic resonance imaging; FLAIR, fluid-attenuated inversion recovery.</p> <p>*Stable doses of corticosteroids include patients not on corticosteroids.</p>	

Table 6.iii.6 Summary of the Proposed RANO Response Criteria

Criterion	CR	PR	SD	PD
T1 gadolinium enhancing disease	None	$\geq 50\% \downarrow$	$< 50\% \downarrow$ but $< 25\% \uparrow$	$\geq 25\% \uparrow^*$
T2/FLAIR	Stable or \downarrow	Stable or \downarrow	Stable or \downarrow	\uparrow^*
New lesion	None	None	None	Present*
Corticosteroids	None	Stable or \downarrow	Stable or \downarrow	NA ^Δ
Clinical Status	Stable \uparrow	Stable or \uparrow	Stable or \uparrow	\downarrow^*
Requirement for response	All	All	All	Any*

Abbreviations: RANO, Response Assessment in Neuro-Oncology; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FLAIR, fluid-attenuated inversion recovery; NA, not applicable
* Progression occurs when this criterion is present.
Δ Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

The primary analysis variables for the study are:

- Percentage change in K_1 , FLT flux and SUV for [¹⁸F]FLT PET/CT
- Assessment of clinical response per the response criteria listed above.
- Survival

Secondary analysis variables are:

- Heterogeneity in K_1 , FLT flux and SUV for [¹⁸F]FLT PET/CT at baseline.
- Comparison of heterogeneity at different times

6.iii.13. DATA REPORTING / REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting are described in Section 6.iii.8. The Principal Physician Investigator is responsible for maintaining complete and timely, HIPAA-compliant electronic records of clinical and image datasets.

6.iii.14. STATISTICAL CONSIDERATIONS

6.iii.14.a. Study Description

This is a study involving [¹⁸F]FLT PET/CT imaging of patients with brain tumors at different times during the course of standard treatment. Thirty patients with brain tumors and who are planning standard therapy with surgical resection, chemotherapy or radiotherapy or any combination, will be enrolled. We will evaluate the values for percentage changes in [¹⁸F]FLT PET measures observed following 1-3 weeks of therapy, shortly after finishing therapy and at time of

recurrence or at one year, whichever comes first and explore associations between [¹⁸F]FLT PET parameters and their changes and clinical outcome.

6.iii.14.b. Power Calculations

For hypotheses 2 and 3, we will use patient survival as our outcome variable and the analysis will be based on the Cox proportional hazards model with adjustment for confounding. We have estimated that with 30 patients our study will have power characteristics presented in Table 6.iii.7. These calculations used a value for the study effect of 0.75.

Table 6.iii.7. Power calculation for measuring response	FU=2 yrs	FU=7 yrs
Power without cofounders	0.79	0.80
Power using cofounders, including FDG PET/CT	0.87	0.83
Effect of 15% measurement error	0.89	0.84

The analysis of data for these hypotheses involves within-subject changes where the variance in values will be less than between-subject FLT results that we have published. Furthermore, this power analysis has assumed that only data from a single volume of interest (VOI) for each subject will be used. In the context of our aims, this could be considered inefficient. In many subjects it will be possible to include data from spatially well-separated and hence substantially uncorrelated sub-regions within large tumors (Spence, 1998) as distinct sampling units for consideration with this hypothesis. Notwithstanding the additional variability that sub-region measurements will have, it is likely that some gains in statistical precision can be realized by this approach. Thus the 90% power estimate is conservative.

Hypothesis 1. Among GBM patients identified as progressing according to Macdonald criteria at 1-month follow-up, the mean FLT flux in true progression cases will be compared to the corresponding mean in the pseudo-progression cases. Preliminary data indicate that 40% of MRI diagnosed progression can be expected to be pseudo-progression. Our data on recurrence vs radionecrosis for KFLT recurrences showed a mean of 0.017 and a standard deviation of 0.005 (Spence, 2006). The mean of the radionecrosis group was 0.004, roughly one-quarter for that for recurrences. Thus, conservatively, assuming that pseudo-progressors showed even a 30% increase over the mean KFLT value, the expected difference between the groups would be 0.017×0.3 or about 0.005. From this, the expected standardized effect is 0.99. Using a 2-sample t-test with significance level of 5% for assessment of the hypothesis, the power of our study is about 95%.

6.iii.14.c. Analysis Plan

Percentage change between pre-treatment and post-therapy measurements will be computed for [¹⁸F]FLT PET/CT measures of transport, K₁, and flux (KFLT). Log transformations will be considered if the rates of change are highly skewed. Outcome analysis studies are directed toward predicting tumor response. In the case of progression, a multivariate logistic regression will be used to produce an analysis of how the combination of standard diagnostics plus the FLT parameters (K₁, KFLT, SUVmean) obtained shortly after therapy can best be combined to

obtain a prognostic variable for determination of progression. Although the prognostic variable will provide a direct assessment of the probability of response for an individual patient, we will also develop a ROC characterization of its diagnostic performance. We fully appreciate that the small number of patients means that this analysis may suffer from a lack of power but it should be useful in planning a larger follow-up if the results are encouraging.

A similar approach will be used for survival analysis.. Here we will use multivariate Cox regression analysis with both baseline and follow-up variables to be considered.

The heterogeneity of the within-tumor proliferation obtained by application of the elliptical heterogeneity measure developed by Eary et al (2008) will be used in a secondary analysis.

6.iii.14.d. Stratification Factors

Not Applicable.

6.iii.14.e. Reporting and Exclusions

Evaluation of Toxicity: All patients will be evaluable for toxicity from their [¹⁸F]FLT PET.

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