

Protocol Title: A Phase II Study of [18F]-FLT for PET imaging of brain tumors in children

NCT Number: NCT01244737

Coordinating Center: Boston Children's Hospital

Sponsor Investigator: Frederick D. Grant, M.D.
Boston Children's Hospital
Division of Nuclear Medicine and Molecular Imaging

Sponsor Co-Investigator: S. Ted Treves, M.D.
Boston Children's Hospital
Division of Nuclear Medicine and Molecular Imaging

Coordinating Center for the Pediatric Brain Tumor Consortium (PBTC):
Operations and Biostatistics Center (OBC) for the St. Jude Children's Research Hospital

Executive Director of OBC/PBTC: Arzu Onar-Thomas, Ph.D
St. Jude Children's Research Hospital
Department of Biostatistics

Investigational Agent

[18F]-FLT: NSC# 743144 / IND# 104365

IND Sponsor: Dr. Frederick Grant at Boston Children's Hospital

¹⁸F-FLT must be acquired from a radiopharmacy that is included in the IND and approved by the sponsor-investigator.

Table of Contents

1	Objectives.....	4
1.1	Hypotheses to be Tested	4
1.2	Research Plan	4
1.3	Investigational Drug Status	5
2	Background and Significance	5
2.1	Pediatric Brain Tumors.....	5
2.2	Positron Emission Tomography	6
2.3	3'-deoxy-3'-18F-fluorothymidine (18F-FLT)	6
2.4	Summary	7
3	Patient Selection and Eligibility:	8
3.1	Clinical Setting:	8
3.2	Subject Identification:	8
3.3	Subject Enrollment:	8
3.4	Enrollment in Multiple Parts of this Study:	8
3.5	Eligibility Criteria.....	8
3.6	Exclusion Criteria	9
3.7	Inclusion of Women and Minorities:	9
4	Subject Enrollment.....	10
4.1	Subject Identification:	10
4.2	Confirmation of Eligibility:	10
4.3	Confirmation of 18F-FLT-PET:	10
4.4	Informed Consent:	10
4.5	Enrollment and Record Keeping:	10
4.6	Study 1.....	11
4.7	Study 2.....	11
4.8	Study 3.....	11
5	PET Imaging Protocols:	12
5.1	Image Acquisition.....	12
5.2	Data Processing (Central Review)	13
5.3	Incidental Findings	13
5.4	PET Quality Assurance	13
6	Safety Considerations.....	14
6.1	Radioactive Safety	14
6.2	Pharmacological Safety	14
7	Drug Formulation.....	15
8	Correlative Clinical Data	15
8.1	Tissue for histopathology	15
8.2	Laboratory Data	15
8.3	Patient outcomes	15
9	Study Calendar	16
10	Measurement of Effect.....	16
11	Adverse Experiences	16
11.1	Evaluation:	16
11.2	Reporting:	16
11.3	Reporting to the FDA:	17
11.4	Reporting to the IRB:	18
12	Data and Safety Monitoring:	18
12.1	Data Recording:	18
12.2	Safety Monitoring Plan	19
12.3	Study Monitor	19
12.4	Pediatric Brain Tumor Consortium (PBTC):	20
13	Regulatory Issues.....	20
13.1	Institutional Reviews	20

13.2	Informed Consent:	20
13.3	Record Keeping and Documentation:.....	20
14	Statistical Considerations and Subject Number:	20
14.1	Study 1:.....	21
14.2	Study 2:.....	21
14.3	Study 3:.....	21
14.4	Assignment of subject numbers:	22
15	Publication of Findings.....	22
16	References	1

1 Objectives

1.1 Hypotheses to be Tested

The objective of this phase II study is to demonstrate the utility of the radiopharmaceutical, 3'-deoxy-3'- ^{18}F -fluorothymidine (^{18}F -FLT), as an imaging agent to assess cellular proliferation for the characterization and evaluation of central nervous system tumors in children. The primary goals of this investigation are to evaluate the use of ^{18}F -FLT-PET for grading and staging brain tumors at diagnosis, for determining if there has been tumor recurrence, and for early assessment of the response to chemotherapy. ^{18}F -FLT tumor uptake will be assessed by positron emission tomography (PET) and ^{18}F -FLT-PET findings will be correlated with histopathology of tissue obtained from surgical resection/biopsy and with patient outcomes. The proposed studies will evaluate ^{18}F -FLT-PET by testing three hypotheses:

1. ^{18}F -FLT uptake is an accurate marker of cellular proliferation and tumor grade for central nervous system tumors in children. This hypothesis will be tested by correlating ^{18}F -FLT uptake with tumor histology in children with newly diagnosed central nervous system tumors who have or will be undergoing surgical resection.
2. ^{18}F -FLT uptake is an accurate marker of cellular proliferation in children with possible recurrent central nervous system tumors, which should help discriminate recurrent tumor from benign scar or inflammation. This hypothesis will be tested in children with a history of treated central nervous system tumor in whom standard imaging has raised concern for tumor recurrence and for whom previously resected tumor is available for histological analysis.
3. Changes in ^{18}F -FLT uptake, as a measure of cellular proliferation, will demonstrate cellular response to chemotherapy in central nervous system tumors in children and will predict clinical outcome. To test this hypothesis, children undergoing chemotherapy for central nervous system tumors will be studied by ^{18}F -FLT-PET *before* chemotherapy and *after* two cycles of chemotherapy. Absolute ^{18}F -FLT uptake and changes in uptake will be correlated with patient outcomes, including disease-free and overall survival.

1.2 Research Plan

The primary objective of this study is to evaluate the utility of ^{18}F -FLT as a PET imaging agent to assess cellular proliferation for the characterization and evaluation of central nervous system tumors in children. Specifically, we will evaluate the use of ^{18}F -FLT-PET for grading and staging brain tumors at diagnosis, for early assessment of the response to chemotherapy, and for determining if there has been tumor recurrence. A secondary objective is to better define the Biodistribution of ^{18}F -FLT administered to children and adolescents. To accomplish the specific aims, the following clinical studies will be performed:

Study 1: In children with newly diagnosed central nervous system tumors, ^{18}F -FLT-PET will be used to assess tumor grade and proliferation. Subjects will be recruited upon presentation to neuro-oncology or neurosurgery for medical care of newly diagnosed central nervous system tumors. ^{18}F -FLT-PET of the brain and/or spine will be performed either prior to surgical excision of the tumor or after surgical biopsy resection leaving substantial (at least half as assessed by the surgeon) tumor mass. Tumor samples will undergo standard histological analysis for pathological diagnosis, as well as quantitative assessment proliferation as assessed by mitotic index for tumor grade and MIB-1 proliferation (Ki67) staining. Qualitative and quantitative measures of tumor uptake of ^{18}F -FLT will be correlated with histological tumor grade and proliferation.

Study 2: In children in whom standard imaging has raised concern for recurrence of a primary brain tumor, ^{18}F -FLT-PET will be used to assess cellular proliferation and determine if ^{18}F -FLT-PET findings can discriminate tumor from benign tissue. Subjects will be identified as a result of follow-up studies, such as brain MRI, that raise concern for possible tumor recurrence. If surgery/biopsy is planned, ^{18}F -FLT-PET of the brain and/or spine will be performed prior to surgical biopsy of the lesion(s) of concern. Tumor samples from current biopsy/resection or if unavailable, tissue from a previous resection/biopsy must be available for histological analysis. As in study 1, surgical specimens will undergo standard histological analysis for pathological diagnosis, assessment of mitotic index for tumor grade, and MIB-1 proliferation (Ki67) staining. Qualitative and quantitative measures of lesion uptake of ^{18}F -FLT will be correlated with proliferative measures and with the final pathological diagnosis.

Study 3: In children with primary central nervous system tumors, ^{18}F -FLT-PET will be used to assess the response to chemotherapy and to evaluate if a given PET response predicts clinical outcome. This study will be limited to children with neuroepithelial brain tumors that will be receiving initial or subsequent therapy with a new regimen of chemotherapy or biologically-based therapy. ^{18}F -FLT-PET will be performed before initiation of therapy and again after completion of two courses of chemotherapy or approximately 40-70 days after start of therapy. Participants will not be enrolled if they are expected to receive concurrent radiation therapy. No tumor specimens need be available for correlation with ^{18}F -FLT-PET findings, but histological analysis can be performed if tumor is available from a prior resection/biopsy. Absolute and changes in qualitative and quantitative measures of tumor uptake of ^{18}F -FLT will be used to assess changes in tumor proliferation in response to chemotherapy. These findings will be compared to other routine imaging studies such as MRI, and will be evaluated as an early predictor of clinical outcomes including duration of disease-free survival and overall survival.

Study 4: Biodistribution Study: In some participants already enrolled in one of the above studies, whole body PET will be performed to assess the biodistribution of ^{18}F -FLT. This study will be performed only in a subset of participants undergoing FLT-PET of the brain for Study 1, Study 2, or Study 3. Participants will undergo whole body PET at three time points: immediately after ^{18}F -FLT administration, immediately after brain imaging, and again 2-5 hours after tracer administration. See Section 5.1.5 for the anticipated additional time required to complete these extra sequences.

1.3 Investigational Drug Status

^{18}F -FLT is an investigational drug. It is used under IND 104365 issued to the sponsor-investigator Frederick D. Grant, M.D. at Boston Children's Hospital, which references IND 071260 issued to the National Cancer Institute. A clinical site cannot participate in this clinical trial until the IND has been amended to include the site and the responsible investigator for the site. ^{18}F -FLT must be acquired from a radiopharmacy that is included in the IND and approved by the sponsor-investigator.

2 Background and Significance

2.1 Pediatric Brain Tumors

Although rare, brain tumors are the second most common malignancy, the most common solid tumor in children, and are the leading cause of death from solid tumors in children. In the United States, 20,500 new malignant tumors of the central nervous system were diagnosed in 2007, and of these, approximately 2,200 were diagnosed in children < 20 years. In 2007, the prevalence of malignant brain tumors in children was approximately 21,000 in the United States.[1]

A wide variety of tumors may occur in the central nervous system, and prognosis is related to tumor type, histological grade, and location. In adults, a large portion of malignant central nervous system lesions may represent metastases of extra-cranial tumors, but this is less common in children. Although some pediatric central nervous system tumors may represent lesions without a primary brain origin, such as lymphomas and leukemia, most malignant pediatric central nervous system tumors have a neuroepithelial origin and over half are glial tumors.[1] Histological grading is a means of predicting biological behavior, and for most neuroepithelial brain tumors, is predictive of patient outcome. The World Health Organization (WHO) classification of tumors provides a grading scale across the range of central nervous system tumors and is the accepted standard scheme by which these tumors are graded. The WHO classification is based a four point scale [1-4] with grade 1 indicating a low proliferative potential and grade 4 signifying mitotically active, malignant tumors with the likelihood of rapid progression and death. Low-grade tumors (grades 1 and 2) are more likely to respond to therapy and may have an indolent course. High grade tumors (grades 3 and 4) are biologically aggressive and have shorter overall survival.[2] More recently, more quantitative methods of assessing tumor proliferation have been used. The Ki67 tumor proliferation index is assessed by immunohistology using the MIB-1 antibody. Studies in brain tumors have shown that an increased Ki67 index is correlated with higher WHO tumor grade and predicts a poorer prognosis.[3, 4] More recently, immunohistological quantification of mitosis has been used to produce a quantitative mitotic index, which also correlates with tumor grade.[5]

Therapy decisions are based on tumor type, grade, and location within the central nervous system. Chemotherapy and radiation can be tailored to tumor characteristics, but despite multimodality therapy, high-grade, aggressive tumors have a poor prognosis. One of the challenges is assessing response to therapy, and in particular the rapid identification of treatment failure. Although tumor progression and growth may be clearly identified, a poor response to therapy may not be identified for many months with anatomic imaging modalities, including MRI. Without identification of non-effective or poorly effective therapy, there is limited opportunity to modify the treatment plan to a potentially more effective therapy.

2.2 Positron Emission Tomography

Positron emission tomography (PET) utilizes radioactive tracers to selectively measure metabolic pathways of interest and thus provides an imaging tool that effectively measures metabolic or proliferative activity in a tumor. Fluorine-18 fluorodeoxyglucose (^{18}F -FDG) has become the most widely used PET imaging agent, and has found particular utility in oncology as it provides images of physiologic function.[6] FDG is an analogue of glucose, and its cellular retention is a function of glucose metabolism governed by hexokinase.[7] ^{18}F -FDG uptake in tumor cells therefore is a reliable indicator of metabolic activity and has proven useful for evaluating many types of malignancies. For example, it is useful in determining the loco-regional extent as well as the presence of distant metastases in cancers of lungs, colon, ovaries, head and neck, breast and pancreatic cancer. The accuracy of ^{18}F -FDG in determining local extent of the disease, lymph node involvement, distant metastases, and post-therapy recurrent/residual tumors has also been established.[6] Other studies have shown that ^{18}F -FDG uptake often declines after successful treatment. [8-10] Thus, during the course of therapy, PET can be a powerful method to assess changes in tumors over relatively short periods of time; while other imaging modalities are not as effective in assessing the success or failure of a therapy because changes in tumor size and contrast enhancement may not occur for several weeks or months into treatment.

^{18}F -FDG-PET, however, has proven less useful in imaging brain tumors.[11-13] An important limitation of ^{18}F -FDG-PET specific to brain tumors is the high level of glucose utilization in the normal brain. The high uptake of ^{18}F -FDG by the normal brain can obscure some malignancies and make it difficult to accurately assess the extent of low-level uptake. [12, 13] Another limitation is that ^{18}F -FDG is not a selective tracer for tumors. As an indicator of glucose uptake, many cell types other than malignant cells can utilize high levels of glucose. For example, macrophages are characterized by high uptake of ^{18}F -FDG and thus inflammatory lesions can demonstrate increased ^{18}F -FDG uptake.[14, 15] Limitations of ^{18}F -FDG-PET are particularly problematic in the evaluation of recurrent brain tumors. In patients with prior surgical or radiation therapy, inflammatory changes and evolving scar tissue may be indistinguishable from recurrent tumor. In a patient with a new or subtle finding on MRI, repeat neurosurgical biopsy may be required to determine if the changes represent benign or recurrent malignant disease. ^{18}F -FDG-PET can sometimes be helpful in this clinical situation, but sensitivity remains limited by physiological uptake in adjacent normal brain and specificity remains limited by uptake at sites of inflammation.

These limitations of ^{18}F -FDG as a cancer imaging agent have led to an investigation of PET tracers that may be more specific for cellular proliferation. For example, labeled thymidine has been used in cell culture and animal studies for years since it is rapidly incorporated into newly synthesized DNA. Pyrimidine analogues mimic physiological nucleosides in terms of cellular uptake and metabolism through the salvage pathway comprising thymidine kinase-1 (TK-1) and deoxycytidine kinase (dCK).[16] dCK is a key enzyme as it catalyzes the phosphorylation of deoxycytidine and comparable analogues, which leads to intracellular trapping of the compound.[16] Cytosolic TK-1 catalyzes the phosphorylation of various pyrimidine analogues to the corresponding monophosphate, resulting in the intracellular trapping of the substrate. TK-1 activity is very low in G_1 cells, increases at the G_1 /early S boundary, and reaches maximum values in late S phase/ G_2 . High levels of TK-1 activity have been reported within rapidly growing cells including breast cancer.[17] Studies have also demonstrated that TK activity increases about 10-fold as cells enter the DNA synthetic phase.[17, 18]

2.3 3'-deoxy-3'- ^{18}F -fluorothymidine (^{18}F -FLT)

Although initial studies of radiolabeled thymidine analogs for PET imaging focused on ^{11}C -labeled thymidine, [19, 20] this agent is not widely used because it is rapidly metabolized, which confounds interpretation of the

images; moreover, the short half-life of ^{11}C (20 min.) restricts its use to those centers with on-site cyclotrons. As a result, attention has been directed primarily to a pyrimidine analog that could be labeled with ^{18}F ($t_{1/2} = 110$ min.) and is resistant to *in vivo* degradation.[21, 22] Research has focused on imaging DNA synthesis with FLT (3'-deoxy-3'-fluorothymidine), an antiviral compound that has been tested in subjects with HIV. [23] ^{18}F -FLT is taken up by cells and phosphorylated by thymidine kinase 1 (TK), but is not incorporated into DNA, which leads to intracellular trapping within the cell.[24] Thus, retention of ^{18}F -FLT within the cell indicates cellular TK activity, a measure of cellular proliferation.

^{18}F -FLT-PET can reveal early and specific changes after treatment of human esophageal squamous cell carcinoma.[25] In canine and human subjects with non-small cell lung cancer, FLT is retained in proliferating tissues by the action of TK and produces high-contrast images of normal marrow and tumors.[26] Vesselle, *et al.* evaluated ^{18}F -FLT-PET for noninvasive assessment of proliferation rates of biopsy-proven, non-small cell lung cancer in 10 subjects.[27] They demonstrated that ^{18}F -FLT-PET may have a useful role in the evaluation of indeterminate pulmonary lesions; in the prognostic assessment of resectable, non-small cell cancer of lung; and possibly in the evaluation of tumor response to chemotherapy. Buck *et al.* evaluated ^{18}F -FLT-PET in thirty subjects with solitary pulmonary nodules.[28] With use of standardized uptake values (SUVs) of ^{18}F -FLT uptake, uptake appeared specific for malignant lesions and may be used for differential diagnosis of solitary pulmonary nodules, assessment of proliferation, and estimation of prognosis. Thus, *in vivo* and *in vitro* studies have shown that FLT-PET can be an accurate method for evaluating malignant disease and for differentiating benign from malignant tissues.

^{18}F -FLT has been a particularly attractive agent for imaging brain tumors because its background activity in the brain is much lower than is seen with ^{18}F -FDG.[29] Spence, *et al.*[30] used dynamic PET and mathematical modeling to estimate transport and retention of ^{18}F -FLT in four glioma patients and observed that, after therapy, ^{18}F -FLT transport decreased, suggesting an observable decrease in cellular proliferation after therapy. This occurred despite an increase in the volume of the 3-D region of ^{18}F -FLT uptake, suggesting that the decreased uptake did not reflect *only* tumor shrinkage. Choi, *et al* [31] compared ^{18}F -FLT and ^{18}F -FDG for PET imaging of brain tumors in 22 patients. In 18 of these patients with isometabolic or hypometabolic lesions on FDG PET, the sensitivity and specificity of FLT-PET was 83% and 83%, respectively. This study also demonstrated a significant difference between the lesion-to-normal tissue ratios in high (5.7 ± 2.2 , $n=7$) and low (2.3 ± 1.1 , $n=5$) grade gliomas ($p < 0.05$). Thus, FLT-PET was of value in making the differential diagnosis of brain tumors that were not evident on FDG-PET. In a similar study, Kim, *et al* [32] compared FLT-PET and FDG-PET in 16 patients with brain tumors. Overall, FLT studies were positive in 22 of 26 lesions (85%) compared to 15 or 26 (58%) with FDG, despite the fact that the overall absolute uptake was lower with FLT than with FDG. They also observed higher uptake of FLT in high-grade (6.07 ± 0.76) versus low-grade (3.11 ± 2.15) gliomas ($p=0.002$), while no significant difference was observed with FDG. Chen, *et al* [33] also reported that FLT was more sensitive than FDG for evaluating recurrent tumors and that it correlated with the Ki-67 index, a histopathological measure of proliferation. Further, early clinical studies in adult patients with central nervous system tumors have suggested that FLT-PET may be more sensitive than FDG-PET in identifying malignant lesions; may provide more accurate information regarding tumor grade; and may be more useful in imaging evaluation of recurrent tumors. Very few children with central nervous system tumors, however, have been included in published studies; nor have any studies assessed the potential effects of age and smaller size on FLT biodistribution. In a clinical study, Choi *et al* [31] included 8 subjects ages 8-13 years, but in none of these subjects was a histopathological measure of proliferation (e.g., the Ki-67 index) reported. Finally, there has been no reported use of FLT-PET to assess pediatric patients with possible recurrence of central nervous system tumors.

2.4 Summary

In summary, ^{18}F -FLT has been investigated as an agent for PET imaging of cellular proliferation to assess tumor grade and viability. It also may be of particular value in assessing the response to non-surgical therapy. Pharmacokinetic studies and tumor imaging studies of ^{18}F -FLT have been reported in adults with a wide variety of tumors. A small number of published studies have reported the use of ^{18}F -FLT in adults with brain tumors, but there very little information is available on the clinical utility of ^{18}F -FLT as a medical imaging agent in children with central nervous system tumors. The goals of this project, therefore, are to assess the utility of FLT-PET in three clinical situations: 1) staging and grading brain tumors at diagnosis; 2) assessing tumor

recurrence; and 3) evaluating response to chemotherapy early in the course of therapy, which may help guide treatment planning. These proposed studies aim to validate the utility of ¹⁸F-FLT-PET to assess cellular proliferation, tumor grade, and response to therapy in pediatric brain tumors. These findings should support efforts to transition ¹⁸F-FLT from experimental drug status to that of a disease-specific PET imaging agent in routine clinical use for evaluating central nervous system tumors in children. An adequate supply of ¹⁸F-FLT will be provided by PETNet, a subsidiary of Siemens Medical Imaging.

3 Patient Selection and Eligibility:

3.1 Clinical Setting:

The defined clinical setting comprises pediatric patients with the diagnosis of central nervous system tumor.

3.2 Subject Identification:

Subjects will be identified at the time of presentation for medical care for a central nervous system tumor. The diagnosis of central nervous system tumor will be made on the basis of clinical and radiological findings. Individuals with metastatic disease or recurrence within the central nervous system (e.g. seeding to the spine) are eligible, but individuals with extra-axial tumors or CNS metastases from non-CNS tumors are not eligible.

All patients meeting the eligibility and exclusion criteria will be offered the opportunity for enrollment. Subjects will be only imaged as part of this study and there is no expected intervention effect. All identified patients meeting inclusion and exclusion criteria will be offered an opportunity to participate in this study. As the proposed research is not a treatment study, no randomization of subjects is required, and all patients entered into the study will undergo the same imaging studies. No control group will be enrolled.

3.3 Subject Enrollment:

Subjects may be enrolled at one of three time points in the clinical course of disease:

3.3.1 Study 1 (New Diagnosis):

Pediatric patients with newly diagnosed primary central nervous system tumors undergoing surgical resection/biopsy within 21 days or who, within the prior 21 days, have undergone resection/biopsy with substantial residual (greater than half as assessed by the surgeon) tumor.

3.3.2 Study 2 (Possible Tumor Recurrence):

Pediatric patients with a history of treated primary central nervous system tumor, in whom standard imaging has raised concern for tumor recurrence. Tumor tissue for histological analysis must be available from a biopsy/resection planned within the next 21 days or from a prior resection/biopsy if no current biopsy material is available.

3.3.3 Study 3 (Response to Therapy):

Pediatric patients with a primary central nervous system tumor who will be starting a new regimen (standard or experimental) of chemotherapy within 21 days and who will not be receiving concurrent radiation therapy.

3.4 Enrollment in Multiple Parts of this Study:

Subjects enrolled in any of the studies (3.3.1, 3.3.2, or 3.3.3) are eligible for concurrent enrollment in the Biodistribution Study. [Determination of whether a participant is asked to do the whole body study depends on patient availability, PET camera availability, travel time after the study, medical stability, ability of the patient to cooperate, etc. It is up to the physician getting consent to decide whether to ask the family to consider participation in the bio-distribution study.](#)

Subjects enrolled with a new diagnosis (3.3.1) will be eligible for imaging at subsequent time points (3.3.2 and 3.3.3). Likewise, a subject enrolled in either 3.3.1 and/or 3.3.3 could be imaged in the future for evaluation of recurrence (3.3.2).

3.5 Eligibility Criteria

3.5.1 Patients should be ≤ 21 years of age at the time of diagnosis.

3.5.2 Patients should be capable of achieving PET imaging without the need for sedation or anesthesia.

3.5.3 Karnofsky Performance Status ≥ 50 for patients' ≥ 12 years of age. For children ≤ 12 years of age, the Lansky play scale $\geq 50\%$ can be substituted (See appendix A).

- 3.5.4 Signed informed consent by subject (age ≥ 18 years) or by parent/guardian (subject age < 18 years). Information will be provided to potential subjects and their parents/guardians (as appropriate) by oral discussion with opportunity for question and answer and the written informed consent document. Subjects less than 18 years of age capable of giving assent will be included in these discussions and will be asked for written assent on the same document as the parents/guardians give consent. If feasible, both parents (or guardians) will be included in these discussions and will be asked to sign the written consent document. If a second parent or guardian is unavailable, this will be explained in writing on the written consent document. If subjects age 18 years or older are unable to provide informed consent, then they will not be enrolled in this study.
- 3.5.5 Patients receiving glucocorticoids and/or anti-seizure medications are eligible for this study.

3.6 Exclusion Criteria

- 3.6.1 Clinically active infection. An active infection may alter the biodistribution of ^{18}F -FLT.
- 3.6.2 Known pregnancy or breast feeding. Pregnant women are excluded as the effects of ^{18}F -FLT on the fetus are not known, and there is the potential for teratogenic or abortifacient effects. Within 48 hours prior to a PET scan, a pregnancy test will be obtained in all female participants of child bearing potential to confirm non-pregnant status. Because there is an unknown, but potential, risk of adverse effects in nursing infants, breastfeeding should be discontinued before the mother receives ^{18}F -FLT
- 3.6.3 Serious intercurrent medical illness
- 3.6.4 Patients requiring emergency surgical intervention that would be inappropriately delayed by FLT-PET imaging.

3.7 Inclusion of Women and Minorities:

Gender and/or minority status will not be used as selection criteria for enrollment in the study. As the proposed research is not a treatment study, no randomization of subjects is required, and all patients entered into the study will undergo the same imaging studies. There is no evidence in the literature that the relationship between FLT avidity and tumor proliferation is altered by gender or minority status.

The reported gender-based prevalence of malignant pediatric brain tumors is 1.4 times more common in males than females. [1] Therefore, we anticipate that subject recruitment will reflect this risk resulting in accrual of more males than females. The minority-based prevalence of malignant brain tumors is 1.3 times more common in whites than in minority populations.[1] Similarly we anticipate subject recruitment that will reflect a patient population adjusted for the increased risk of malignant pediatric brain tumors in the non-minority population.

The maximum sample size for this trial is 75 patients with the likelihood that it will be smaller since patients are allowed to enroll in multiple studies. The table below reflects the anticipated enrollment figures by gender and race/ethnicity:

Accrual Targets					
Ethnic Category	Sex/Gender				
	Females		Males		Total
Hispanic or Latino	4	+	5	=	9
Not Hispanic or Latino	29	+	37	=	66
Ethnic Category: Total of all subjects	33 (A1)	+	42 (B1)	=	75 (C1)
Racial Category					
American Indian or Alaskan Native	0	+	0	=	0
Asian	2	+	2	=	4
Black or African American	5	+	6	=	11
Native Hawaiian or other Pacific Islander	0	+	0	=	0
White	26	+	34	=	60
Racial Category: Total of all subjects	33 (A2)	+	42 (B2)	=	75 (C2)

(A1 = A2)

(B1 = B2)

(C1 = C2)

Specific outreach activities into the community will be not be undertaken in this study (1) because of the nature of central nervous system tumor; and (2) because prospective study subjects must present with an active and acute medical condition.

4 Subject Enrollment

4.1 Subject Identification:

Subjects will be identified by one or more investigators in neuro-oncology, neurosurgery, or radiology at a participating clinical site. An investigator will contact the principal investigator or designee to confirm subject eligibility for one of the three studies. For potential subjects at a PBTC site the Principal investigator or designee will confirm subject eligibility for one of the three studies.

4.2 Confirmation of Eligibility:

The principal investigator or designee will confirm patient eligibility for one of the three studies (Section 3.3) and will confirm that the patient meets each inclusion (Section 3.5) and exclusion criteria (Section 3.6).

4.3 Confirmation of ¹⁸F-FLT-PET:

Using established procedures, the site investigator will ascertain availability of ¹⁸F-FLT and availability of imaging time in the Division of Nuclear Medicine / PET at the appropriate imaging facility. Imaging will be performed within the prescribed window of time before or after any scheduled surgical procedure or before initiation of chemotherapy. Availability of ¹⁸F-FLT from the commercial supplier will depend on the current manufacturing schedule and PET imaging may be constrained by the clinical imaging schedule in the PET department. Subject enrollment will not occur if PET imaging cannot be performed.

4.4 Informed Consent:

- 4.4.1 Informed written consent (and assent if appropriate) will be obtained by a site investigator after confirmation that ¹⁸F-FLT-PET can be scheduled and performed.
- 4.4.2 For subjects age 18y or greater, informed written consent will be obtained.
- 4.4.3 For subjects less than age 18y, informed written consent will be obtained from parents/guardians. When appropriate, written assent will be obtained from subjects less than age 18 y old.
- 4.4.4 The original, signed, written consent/assent will be maintained by the PBTC local site investigator following local institutional procedures. A copy of the signed consent/assent will be faxed to the OBC from the PBTC member's site.

4.5 Enrollment and Record Keeping:

PBTC Member Sites:

Informed consent must be obtained prior to patient registration. Patients must be registered prior to any protocol treatment. Patient registration is only available to authorized personnel using the PBTC automated registration system. The registration procedures are available in the PBTC CRA manual, which is posted on the PBTC member's website. The PBTC Protocol Coordinator may also be contacted at 901-595-3783 for assistance in the registration process

Reservations may also be made through the registration system providing time to assess the patient's eligibility. Reservations will be held for a maximum of 7 calendar days by which time the patient must have been registered on study. The patient's reservation should be canceled as soon as it is determined that the patient is not eligible, that the family/patient has decided not to consent to participation, or the FLT scan will not be able to be performed within the prescribed time.

Boston Children's Hospital:

For each subject, upon enrollment, a study file will be started. For each subject, the study file will include the signed written consent /assent form, completed intake/eligibility screening form, and any clinical information necessary to document eligibility.

4.6 Study 1

In children with newly diagnosed central nervous system tumors, typically established as a likely neuroepithelial brain tumor on MRI, use ^{18}F -FLT-PET to assess tumor grade and proliferation:

- 4.6.1 ^{18}F -FLT-PET of the brain and/or spine will be performed within 21 days before surgical excision/biopsy of the tumor or within 21 days after surgical resection/biopsy leaving substantial (at least half as assessed by the surgeon) residual tumor.
- 4.6.2 Surgical resection or biopsy will be performed as part of usual clinical care.
- 4.6.3 Tumor samples will undergo standard histological analysis for pathological diagnosis, assessment of mitotic index for tumor grade, and MIB-1 proliferation (Ki67) staining.
 - PBTC sites will provide Boston Children's Hospital either unstained slides or slides stained for Ki67 quantitative MIB along with the original pathology report.
- 4.6.4 Qualitative and quantitative measures of tumor uptake of ^{18}F -FLT will be correlated with histological tumor grade and proliferation.

4.7 Study 2

In children in whom standard imaging has raised concern for recurrence of a neuroepithelial central nervous system tumor, use ^{18}F -FLT to assess cellular proliferation and determine if ^{18}F -FLT-PET can discriminate tumor from benign tissue. This study will be performed in children with a history of neuroepithelial central nervous system tumor in whom there is concern for tumor recurrence.

Tumor issue for histological analysis must be available from a prior resection/biopsy or from a biopsy/resection planned within the next 21 days.

- 4.7.1 Any surgical biopsy will be performed only as part of usual and standard clinical care.
- 4.7.2 Tumor samples will undergo histological analysis for pathological diagnosis, assessment of mitotic index form tumor grade, and MIB-1 proliferation (Ki67) staining.
 - PBTC sites will provide Boston Children's Hospital either unstained slides or slides stained for Ki67 quantitative MIB along with the original pathology report.
- 4.7.3 Qualitative and quantitative measures of lesion uptake of ^{18}F -FLT will be correlated with proliferative measures. Correlation also will be made with the final pathological determination of recurrent tumor or benign tissue.

4.8 Study 3

In children with neuroepithelial central nervous system tumors, use ^{18}F -FLT-PET to assess the response to chemotherapy and evaluate if this response predicts clinical outcome. This study will be performed in children receiving a new regimen of chemotherapy or biologic-based therapy for treatment of a central nervous system tumor. Children who are expected to receive concurrent radiation therapy will not be enrolled.

- 4.8.1 Patients will undergo ^{18}F -FLT-PET of the brain and/or spinal column at two time points:
 - 4.8.1.1 *Prior to initiation of chemotherapy, if patients underwent ^{18}F -FLT-PET imaging prior to surgical biopsy, the pre-chemotherapy ^{18}F -FLT-PET still will be repeated to take into account changes in tumor architecture or proliferation that may have occurred in response to neurosurgical intervention.*
 - 4.8.1.2 *The second ^{18}F -FLT-PET will be performed after completion of the second course of chemotherapy (typically less than two months -40-70 days- after start of chemotherapy), but before the third course of chemotherapy, before any additional surgery, and before any radiotherapy.*
- 4.8.2 Qualitative and quantitative measures of tumor uptake of ^{18}F -FLT will be determined and used to assess tumor proliferation measures after chemotherapy. Tumor proliferation after chemotherapy will be assessed as an early predictor of clinical outcomes including duration of disease-free survival and duration of survival.
- 4.8.3 Subjects enrolled in Study 2 may be enrolled in the Study 3 only if there is sufficient residual tumor for imaging.

4.9 Evaluable Participants: Participants shall be considered evaluable if the following criteria are met:

- 4.8.1. The participant (or parent/guardian) has not withdrawn consent
- 4.8.2. The participant has not been found to be ineligible after being enrolled in the study.

4.8.3. All study requirements have been met. This includes:

4.8.3.1. The FLT-PET has been performed and is technically adequate for analysis.

4.8.3.2. Pathologic evaluation confirms the diagnosis of a brain tumor. For example, a participant may be enrolled appropriately in Study 1 prior to surgery, and if the final pathologic diagnosis does not demonstrate a neuroectodermal brain tumor (e.g. lymphoma or infection), then that participant will not be considered evaluable.

4.8.3.3. Histological analysis, including Ki67 (MIB) evaluation, is completed and technically adequate for analysis.

4.8.3.4. Long-term follow-up and survival data collection have been completed.

4.10 Study Completion

4.10.1. Study Intervention and Active Follow-up: As part of the Safety Monitoring Plan, blood tests will be performed within one week after the FLT-PET scan. Once these post-scan blood test results are obtained, a participant will be deemed to have ended study intervention and active follow-up.

4.10.2 Long-Term Follow-up and Survival: Long-term follow-up and survival data collection will be performed for two years (or until death) after the last FLT-PET scan, at which time the participant will be deemed to have completed all study requirements and will become an Inactive Study Participant.

4.10.3. Inactive (Off-Study): A participant will be deemed to be an Inactive Study Participant (Off-Study) for any of the following reasons:

4.10.3.1. Participant death before completion of active follow-up.

4.10.3.2. Participant (or parent/guardian) has withdrawn consent

4.10.3.3. Participant is found to be ineligible after being enrolled in the study..

4.10.3.4. Participant is determined to be not evaluable (section 4.9) or lost to follow-up

4.10.3.5. Participant has completed all study requirements

4.9 PET Imaging Protocols:

Subjects will undergo PET imaging with ^{18}F -FLT. For each study, all PET imaging will be performed after administration of a single dose of ^{18}F -FLT. Participants must be able to cooperate with required imaging without anesthesia or sedation.

4.10 Image Acquisition

4.10.1 Patient Preparation: No special patient preparation is required. Patients do not need to be fasting (unlike ^{18}F -FDG) and can receive intravenous fluids as indicated by clinical indication.

4.10.2 ^{18}F -FLT Dose: Based on prior literature and radiation dosimetry calculations (Section 8), an ^{18}F -FLT dose of 0.15 mCi/kg, with a maximum dose of 10 mCi, will be used for all studies. This is similar to the dose used for FDG-PET imaging in pediatric patients. Based on the specific activity of ^{18}F -FLT (>200 Ci/mmol), the mass of ^{18}F -FLT administered will be approximately 180 nanogram/kg (maximum 12 microgram).

4.10.3 ^{18}F -FLT Administration: The ^{18}F -FLT is administered through an intravenous catheter as per standard PET procedures.

4.10.4 Brain PET Acquisition: In all subjects, at 30 minutes post-administration, a brain and/or spine PET scan will be acquired in 3-D mode with a 5-10 min emission scan at each bed position, including all areas of suspected tumor (brain, spine, or brain and spine). For attenuation correction, a low-dose CT will be acquired of the imaged region, per standard PET/CT procedures.

4.10.5 Biodistribution PET Acquisition (12 subjects): In the twelve subjects undergoing the biodistribution study, PET of the head and torso (field of view extending from top of head to the mid-thighs) will be performed at three time points: (1) immediately after ^{18}F -FLT administration, (2) at approximately one hour after ^{18}F -FLT administration, after completion of the brain PET, and (3) at 2-5 hours after ^{18}F -FLT administration. Biodistribution PET may be acquired in 2-D mode with a 3 min emission scan at each bed position or in 3-D mode with a 2 min emission scan at each bed position. For attenuation correction, low-dose CT scans will be acquired of the imaged regions, per standard PET/CT procedures.

Scan	Approximate start time (injection time 0:00)	Approximate Length of Scan
Full-body	0:05	20 minutes
Brain	0:30	15 minutes
Full-body	1:00	20 minutes
Full-body	2:00-5:00	20 minutes

4.10.6 PET Processing: All PET scans are reconstructed using an OSEM algorithm with CT-based attenuation correction.

4.10.7 MRI for Correlation: An MRI of the involved regions (brain and/or spine) is required for image co-registration and anatomic correlation. These should be the most recent MRI studies of the involved regions that were performed with and without gadolinium contrast. An additional brain or spine MRI does not need to be performed specifically for this protocol.

4.10.8 Image submission: FLT-PET and recent MRI will be submitted through the PBTC for central review. The reconstructed PET data and correlative MRI scans should be submitted in standard DICOM format.

4.11 Data Processing (Central Review)

4.11.1 Co-registration with MRI: Brain and/or spine PET images will be co-registered to the most recent previously acquired MRI using visual analysis, which may be assisted by commercially available fusion software (Seimens Fusion 3D or Hermes Medical Solutions).

4.11.2 Image Analysis: Assessment of FLT uptake will be performed independently by at least two investigators experienced in image interpretation who will then create summary results by consensus. All central reviews will be performed in the Neuroimaging center (NIC) of the PBTC.

4.11.3 Blinding: Analysis will be performed in a masked (blinded) manner with the evaluator unaware of the results of other imaging studies, histopathological studies, or patient outcome.

4.11.4 Tumor Delineation: 3D regions of interest will be drawn around the tumor. In addition, an analogous ROI will be drawn in normal brain background and about the whole brain for comparison.

4.11.5 Quantitative Assessment: On each brain PET scan, tumor uptake of ¹⁸F-FLT will be assessed in two ways:

4.11.5.1 Abnormal uptake graded on a 4-point scale of uniformity of uptake

4.11.5.2 Determination of standard uptake values (SUV's) for which tumor, tumor-to-background, and tumor-to-whole brain ratios will be determined using maximal and mean SUV's.

4.11.6 Biodistribution: In those studies including torso imaging, FLT biodistribution will be assessed by semi-quantitative region-of-interest analysis of sites with substantial tracer uptake.

4.12 Incidental Findings

As the significance of abnormal findings may not be known, findings of FLT-PET imaging are not intended to be used for patient management. If a radiology report describing the findings of the FLT-PET will be available to treating physicians, the report should include a disclaimer stating that the study is investigational and not intended for clinical use. However, it is possible that in occasional subjects, an incidental finding will suggest an unsuspected site of disease or other clinically important finding. As this could alter patient evaluation and management, the treating/referring physician will be informed of any significant findings at the time of PET acquisition or analysis.

4.13 PET Quality Assurance

The proposed sites of this multi-center trial are part of the current PBTC PET Quality Control Program, which is applicable to all F-18 labeled PET radiopharmaceuticals. Consistency of PET data is maintained by adherence to a standard quality assurance program with daily blank scans and quarterly normalization, calibration, and preventive maintenance. Each site must complete a quarterly report that includes a PET Quality Assurance data form, which is submitted to the Neuroimaging Center of the PBTC. Summary reports of the PET QA program are generated on an ongoing basis.

As part of the PET Quality Control program, each of the institutions has participated in the analysis of a uniform PET phantom that was deployed to each site. This activity was performed with Paul Kinahan, PhD

(Univ. Washington) and Laurence Clarke, PhD (NIH) and was overseen in collaboration with the Society of Nuclear Medicine and Molecular Imaging (SNMMI) and the American Association of Physicists in Medicine (AAPM). These findings were published as a peer-reviewed article.[34] In the future, the Neuroimaging Center of the PBTC may design other quality assurance initiative that will deploy a different PET phantom for evaluation at each site. It is anticipated that all sites will participate in this quality control initiative.

5 Safety Considerations

5.1 Radioactive Safety

¹⁸F-FDG-PET has been used safely in adult human subjects and has been shown to be effective in both in vivo and in vitro studies as discussed in the Background. In this study, subjects will receive ¹⁸F-FLT at an intravenous dose of 0.15 mCi/kg. This will give an effective dose (i.e. whole body) of 5.6 to 8.8 mSv depending upon body size. The normal liver and bone marrow show relatively homogenous uptake of ¹⁸F-FLT and it undergoes renal excretion. The calculated effective radiation dose (whole body) and the dose to the critical organ (bladder) are similar to that associated with FDG-PET (below). FLT dosimetry is calculated using OLINDA software (Vanderbilt University) [35] and residence times from Vesselle et al. [36] FDG dosimetry is calculated from ICRP Report 80. [37]

Comparative dosimetry for FLT and FDG[36]- [37]

*In both cases, consider 0.15 mCi/kg (5.55 MBq/kg)

Subject Age	1 Year	5 Year	10 Year	15 Year
Mass (kg)	9.8	19.0	32.0	55.0
Admin Act mCi (MBq)	1.46 (53.8)	2.97 (109.9)	4.98 (184.3)	8.52 (315.2)
FLT*				
Bladder Wall Dose (mGy)	23.0	25.3	27.5	31.2
Effective Dose (mSv)	6.8	5.6	8.8	7.3
FDG**				
Bladder Wall Dose (mGy)	31.7	35.2	51.6	66.2
Effective Dose (mSv)	5.1	5.5	6.6	7.9

5.2 Pharmacological Safety

In vitro: During *in vitro* studies of non-radioactive FLT, chromosomal abnormalities were seen when cells were cultured with very high doses of FLT for more than 12 hours. This effect on chromosomes diminished with longer incubation and was not seen with lower doses of FLT. The levels of ¹⁸F-FLT that will be used in this study are 200 times less than the lowest level that caused chromosome damage in *in vitro* laboratory tests. However, similar studies have not been performed with ¹⁸F-FLT.

In vivo: Non-radioactive FLT (also called MIV 310 or alovudine) has completed phase II clinical trial in patients with HIV. FLT was given to a total of 140 HIV-infected patients in 6 well-documented clinical studies. The patients were treated with FLT as the only antiviral treatment for up to 16 weeks. At daily dose of 7.5 mg for 4 weeks, FLT was generally well tolerated and did not result in any serious events. [38] For this study, ¹⁸F-FLT dose will be adjusted based on patient weight. Prior human studies in adults with FLT-PET have not shown any adverse effects at doses similar to that proposed for this study (0.15 mCi/kg). Based on the specific activity of ¹⁸F-FLT (>200 Ci/mmol), the mass of ¹⁸F-FLT administered (180 nanogram/kg) for PET imaging is

approximately 500 times less than the dose of non-radioactive FLT administered for therapeutic effect (approximately 0.1 mg/kg). Thus, negligible pharmacological effect or risk is anticipated.

6 Drug Formulation

The radiopharmaceutical, 3'-deoxy-3'-¹⁸F-fluorothymidine (¹⁸F-FLT) will be used for all imaging studies. The attached IND application includes chemistry, manufacturing, and control data. The compound has a physical half-life of 110 minutes. ¹⁸F-FLT must be acquired from a radiopharmacy that is included in the IND and approved by the sponsor-investigator. . On an as needed basis, FLT will be ordered, delivered, and disposed of using the same procedures as for other radiopharmaceuticals used in the Division of Nuclear Medicine at the participating site. Drug reconstitution is not needed. Administered doses are confirmed by standard operating procedure using a dose calibrator. FLT will be administered intravenously at a dose of 0.15 mCi/kg, with a maximum dose of 10 mCi as described in the PET Imaging Protocol (section 4.9).

7 Correlative Clinical Data

7.1 Tissue for histopathology

Subjects will undergo surgery (biopsy or gross total resection) as indicated for usual clinical care.

7.1.1 Tissue specimens will be processed and analyzed in the local pathology department per usual routine.

7.1.2 Tumor samples will be analyzed for MIB-1 proliferation by Ki67staining. Ki-67 staining may be performed at the local institution if Ki-67 staining is clinically indicated. Stained (1) or unstained (2-4) slides appropriate for quantitative MIB-1 evaluation as well as the associated local pathology report will be sent to Boston Children's Hospital for quantitative analysis. PBTC sites, please add Briana Sexton-Stallone (briana.sexton-stallone@childrens.harvard.edu) in the email notification when completing the FedEx shipment.

Briana Sexton-Stallone
Department of Radiology LC-
Boston Children's Hospital
300 Longwood Avenue
Boston, MA 02115)
Telephone: 617-355-7719
Fax 617-730-0620

All tumor grading and Ki-67 scoring will be performed in the Department of Neuropathology at Boston Children's Hospital using standard CLIA-certified methodology. The division has a quality control program for all histological scoring procedures.

7.2 Laboratory Data

As part of the Safety Monitoring Plan, subjects will have baseline laboratory assessment within two weeks prior to ¹⁸F-FLT administration followed by another laboratory assessment performed prior to surgery or initiation of therapy, but within one week after ¹⁸F-FLT administration. These laboratory tests may be obtained as part of routine clinical care of these patients, but when results are not available, the tests will be requested as part of this protocol. Laboratory assessment before and after ¹⁸F-FLT administration will include the following tests:

7.2.1 Serum electrolytes: sodium (Na), potassium (K), bicarbonate, and chloride(Cl)

7.2.2 Tests of renal function: blood urea nitrogen (BUN), serum creatinine (Cr)

7.2.3 Tests of liver function (bilirubin, ALT, AST, and alkaline phosphatase)

7.2.4 Complete blood count (CBC), including values for hematocrit, hemoglobin, platelet count, and white blood cell count with differential.

7.3 Patient outcomes

In all subjects, clinical outcome measures including progression-free survival and median survival time will be ascertained by follow-up chart review and by contact with the referring/treating neuro-oncologists and neurosurgeons. Patient status from PBTC member sites will be recorded in the PedBraTum/RDC electronic case report form.

8 Study Calendar

	Within 14 days before study	Within 48 h before study	Day of Study	Within 1 week after study	Within 2 years after study
Laboratory tests per Safety Monitoring Plan	X			X	
Pregnancy test (females ≥ 11 y)		X			
Focused Physical Exam			X		
Vital Signs			X		
^{18}F -FLT PET of brain/spine			X		
Long-term monitoring					X

9 Measurement of Effect

The aims of this study are to evaluate the diagnostic performance of the radiopharmaceutical, 3'-deoxy-3'- ^{18}F -fluorothymidine (^{18}F -FLT). There is no therapeutic intent or expected therapeutic effect with the proposed studies.

10 Adverse Experiences

10.1 Evaluation:

CTCAE term (AE description) and grade:

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

All adverse experiences which occur within 1 day (24hrs) of ^{18}F -FLT administration will be recorded, including severity of reaction (i.e., mild, moderate, severe), relationship to study drug (i.e., probably related, unknown relationship, definitely not related), date and time of administration of test medications and all concomitant medications, and medical treatment provided. The site investigator will be responsible for evaluating all adverse events to determine whether criteria for "serious" and "unexpected" are present.

10.2 Reporting:

Boston Children's Hospital

For all severe adverse clinical experiences related to drug administration, details about the duration and intensity of each episode, the action taken with respect to the drug, and the patient's outcome will be recorded on the Adverse Event Report form. The site investigator will evaluate each adverse experience for its relationship to the drug and for its seriousness.

PBTC Member Sites:

At each participating clinical center, the respective principal investigator and study staff are responsible for reporting adverse events centrally to the PBTC via electronic data entry and the completion of the AdEERS report. AdEERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. AdEERS provides a copy feature for other e-mail recipients.

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to the PBTC Operations Office at 901-595-3783. Once Internet connectivity is restored, the 24-hour notification phoned in or faxed must be entered electronically into AdEERS by the original submitter at the site.

For this study, a serious adverse event is defined as any untoward medical occurrence that meets the reporting criteria noted in the table below. All toxicities which meet the criteria below should be reported via AdEERS.

Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of an Investigational Agent/Intervention ^{1, 2}

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

- 1) Death
- 2) A life-threatening adverse event
- 3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
- 4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- 5) A congenital anomaly/birth defect.
- 6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon 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. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the sponsor via AdEERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour; 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via AdEERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

¹Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

² For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

10.3 Reporting to the FDA:

10.3.1 The Principal Investigator at Boston Children's Hospital is responsible for reporting Adverse Experiences to the FDA.

10.3.2 A written report will be made to the FDA within 15 days of knowing of any adverse event that is (i) associated with the use of the drug and (ii) serious and unexpected.

10.3.3 A written report will be made to the FDA within 15 days of knowing of any finding from tests in laboratory animals that suggest a significant risk for human subjects

10.3.4 In addition to a written report, a telephone or facsimile report will be made to the FDA as soon as possible, but no later than 7 calendar days after knowing any adverse event that is (i) associated with the use of the drug; (ii) unexpected; and (iii) life-threatening or fatal.

10.3.5 Serious adverse events in which a causal relationship between the drug and the event can be ruled out will be reported at the time of submission of the annual report to the FDA.

10.4 Reporting to the IRB:

At each participating clinical center, adverse events will be reported to the local IRB by the respective principal investigator and study staff at the site,

The following will be reported to the IRB within 72 hours of being known:

10.4.1 Any death of a research subject thought to be related or possibly related to research associated interventions.

10.4.2 Adverse events that are also unanticipated problems involving risks to subjects or others.

10.4.3 Unanticipated events that do not involve actual harm to subjects or others, but which place research subjects or others at risk of harm that was not previously anticipated.

11 Data and Safety Monitoring:

11.1 Data Recording:

PBTC member sites will submit all pre-treatment, on-study and off-treatment data as well as patient response data via the electronic data collection screens using the PBTC PedBraTum RDC database. Clinical data on patients accrued to this protocol will include the items noted below. For assistance, contact the PBTC Protocol Coordinator listed on the PBTC N12 webpage. For subjects enrolled at Boston Children's Hospital, clinical record forms will be completed on each subject and maintained in a secure file in the Division of Nuclear Medicine.

11.1.1 General/Clinical Data

- Referring investigator
- Date of birth, age at diagnosis
- Dates of any surgery since diagnosis of brain tumor
- Dates of chemotherapy administration within prior six months

11.1.2 Imaging Studies

11.1.2.1 *For each imaging study the following information should be submitted for central review*

- Date of Study
- Type of study submitted (MRI, FLT-PET)

11.1.2.2 *For each brain FLT-PET the following information should be submitted for central review:*

- Participant weight(kg)
- Participant height (cm)
- Administered Dose (mCi) of ^{18}F -FLT
- Injection time (hh:mm)
- Scan start time (hh:mm)
- Scan end time (hh:mm)
- Notes

11.1.2.3 *For each series of Biodistribution FLT-PET, the following information should be submitted for central review:*

- Participant weight (kg)
- Participant height (cm)
- Administered Dose (mCi) of ^{18}F -FLT
- Injection time (hh:mm)
- Scan start time (hh:mm)
- Scan end time (hh:mm)
- Notes

11.1.3 Histopathology

The results of each histological study will be recorded/reported, including report of standard histological interpretation for pathological diagnosis

11.1.4 Safety Monitoring Data

11.1.4.1 *Physical findings and vital signs obtained as part of the safety monitoring plan before, during and after ^{18}F -FLT administration.*

11.1.4.2 *Clinical laboratory data obtained as part of the safety monitoring plan.*

11.1.5 Clinical Outcome: Clinical outcome data will be collected from the medical record and contact with referring investigators for up to two years from the date of the FLT-PET.

11.2 Safety Monitoring Plan

This Safety Monitoring Plan has been designed with the advice and approval of the FDA and is designed to monitor for both immediate and delayed adverse events.

11.2.1 Immediate:

Surveillance for immediate unexpected adverse events will include monitoring vital signs before, during, and after FLT administration. A focused physical examination will be performed before FLT administration and after completion of imaging.

11.2.2 Delayed:

Monitoring for delayed unexpected adverse events will utilize (1) laboratory testing within one week of ^{18}F -FLT administration and (2) surveillance by the referring/treating neurooncologist throughout the course of each subject's disease.

11.2.3 Laboratory Surveillance

For comparison, subjects will be required to have baseline laboratory assessment within two weeks prior to ^{18}F -FLT administration followed by another laboratory assessment performed prior to surgery or initiation of therapy, but within one week after ^{18}F -FLT administration. These laboratory tests may be obtained as part of routine clinical care of these patients, but when results are not available, the tests will be requested as part of this protocol.

Laboratory assessment before and after ^{18}F -FLT administration will include the following tests:

11.2.3.1 *Serum electrolytes: sodium (Na), potassium (K), bicarbonate, and chloride (Cl)*

11.2.3.2 *Tests of renal function: blood urea nitrogen (BUN), serum creatinine (Cr)*

11.2.3.3 *Tests of liver function (bilirubin, ALT, AST, and alkaline phosphatase)*

11.2.3.4 *Complete blood count (CBC), including values for hematocrit, hemoglobin, platelet count and white blood cell count with differential*

11.2.4 Follow-up Surveillance

Throughout the course of their disease, each subject will have repeated and frequent contact with neuro-oncologists and neurosurgeons, which will facilitate detection of delayed, unexpected adverse events. The referring/treating neuro-oncologists and neurosurgeons are active sub-investigators of this protocol, and if any adverse event is identified, they will inform the Sponsor-Investigator.

11.3 Study Monitor

The study design is not investigating a therapeutic effect. There is no endpoint assessment and there is no control population. To fulfill the requirement of the Sponsor-Investigator to monitor the study's progress, a Study Monitor within the Children's Hospital Office of Clinical Investigation will provide on-going monitoring of data stored for and by Boston Children's Hospital to ensure the protection and safety of all human subjects involved in this investigation, as well as to ensure the quality and integrity of data resulting from this investigation. The Sponsor will ensure the study and subject records are adequately monitored as deemed necessary. For patients enrolled at Boston Children's Hospital, the monitor will perform a source document review to ensure that recorded information and data is consistent with original source documents.

Based on the information provided during each monitoring visit, and as directed by the sponsor, the following items will generally be monitored:

11.3.1 Compliance with the IND and IRB-approved protocols.

11.3.2 Event reporting (adverse, unanticipated, deviation) is accurate, complete, and reported to the IRB, sponsor-investigator, and FDA in a timely manner.

11.3.3 Regulatory documentation is accurate, complete, and current.

11.3.4 The monitor may request to review additional items.

11.4 Pediatric Brain Tumor Consortium (PBTC):

The PBTC Protocol coordinator will monitor data remotely for the participating PBTC sites. The PBTC will be responsible for monitoring enrollment of patients treated at PBTC sites and as needed, to generate queries to notify sites of incomplete data. PBTC meeting book reports will summarize the PBTC participation in the study and will include updated information on the conduct of the trial, as provided by the protocol PI.

Total enrollment on this study will be monitored to ensure adequate and timely accrual to address the research questions. If accrual lags and a feasible corrective action plan is not implemented within three months of identifying the problem, the PBTC will cease participation in the study.

12 Regulatory Issues

12.1 Institutional Reviews

This study will be reviewed and approved by an IRB before the study is initiated, and in a manner consistent with the requirements of 21 CFR part 50 (Protection of Human Subjects). Any changes to the protocol must be approved by the IRB at Children's Hospital Boston before implementation and approved changes will be disseminated to all investigators by the Principal Investigator. The PBTC will be responsible for providing the amended protocol to the participating consortium sites for local review and approval.

12.2 Informed Consent:

All subjects (or parent/guardian, as appropriate) will provide informed consent before being enrolled into this study. Subjects age less than 18 y of age will provide assent, if appropriate. Information will be provided to potential subjects and their parents/guardians (as appropriate) by oral discussion with opportunity for question and answer and the written informed consent document. Subjects less than 18 years of age capable of giving assent will be included in these discussions and will be asked for written assent on the same document as the parents/guardians give consent. If feasible both parents (or guardians, if applicable) will be included in these discussions and will be asked to sign the written consent document. If a second parent or guardian is unavailable, this will be explained in writing on the written consent document. If subjects age 18 years or older are unable to provide informed consent, then they will not be enrolled in this study.

The consent form attached to this protocol will be provided to each subject (or parent/guardian) and subjects will be provided with the information necessary to make an informed decision about participation in this study. No part of the study will be initiated before the subject (or parent/guardian) provides signed written consent on the attached consent form. The individual obtaining consent will sign the consent form. The original consent form will be maintained in the research file at the participating site, and a copy of the signed form will be provided to the subject.

12.3 Record Keeping and Documentation:

Records and documentation described in this protocol will be maintained by the Principal Investigator and by the OBC for the PBTC. Records will be retained as indicated by federal or state regulation and institutional policies. Original medical images will be maintained in a retrievable DICOM electronic format at the clinical site that performed the imaging study. Imaging studies may be stored in a radiology or nuclear medicine PACS or other electronic archive as established by local guidelines and practice. Original medical records will be maintained in paper or retrievable electronic format as established by institutional guidelines at each clinical site.

13 Statistical Considerations and Subject Number:

Each of these three studies is a diagnostic imaging study to assess the utility of ¹⁸F-FLT in the evaluation of pediatric brain tumors. The study design is not studying a therapeutic effect, and no control subjects are included. Several statistical approaches will be utilized for assessing the diagnostic performance of ¹⁸F-FLT-PET.

13.1 Study 1:

Children with newly diagnosed central nervous system tumors, assess the utility of ^{18}F -FLT-PET to assess tumor grade and proliferation:

^{18}F -FLT-PET of the brain and/or spine will be performed prior to surgical excision of the tumor. Tumor samples will undergo standard histological analysis for pathological diagnosis, qualitative assessment of tumor grade (WHO tumor grade I-IV), mitotic index for quantitative assessment of tumor grade, and MIB-1 proliferation (Ki67) staining.

Quantitative measures of tumor uptake of ^{18}F -FLT will be compared between patients with different tumor grade using Wilcoxon rank-sum test, and will be correlated with proliferation (Ki67 staining) using the Spearman rank order correlation coefficient (ρ). In addition, patients will be divided into subgroups based on the presence or absence of ^{18}F -FLT uptake; these subgroups will then be compared in terms of Ki67 proliferation with the nonparametric Wilcoxon rank-sum test. Based on prior literature, which suggests a moderate positive correlation of 0.70 between ^{18}F -FLT uptake and tumor grade among similar tumor types, sample size calculations indicate that 15 evaluable subjects will be sufficient to attain 85% power ($\beta=0.15$, 2-tailed $\alpha=0.05$) to demonstrate a significant correlation of this magnitude.

13.2 Study 2:

Children in whom standard imaging has raised concern for recurrence of a central nervous system tumor, use ^{18}F -FLT to assess cellular proliferation and determine whether ^{18}F -FLT-PET can discriminate recurrent tumor from benign tissue:

^{18}F -FLT-PET of the brain and/or spine will be performed prior to surgical excision/biopsy of the tumor. Tumor samples from current biopsy/resection whenever possible or if unavailable, tissue from a previous surgery/biopsy, will undergo standard histological analysis for pathological diagnosis, qualitative assessment of tumor grade (WHO tumor grade I-IV), quantitative assessment of mitotic index for tumor grade, and MIB-1 proliferation (Ki67) staining.

Median uptake will be compared between those subjects with and without recurrence using the nonparametric two-sample Wilcoxon rank sum test with a two-tailed significance level of 0.05. Assuming that there is an 80% likelihood that uptake in non-tumor lesions will be lower than uptake in tumor lesions, power analysis indicated that 30 evaluable subjects would provide 80% power to detect a difference of 30% in each ^{18}F -FLT-PET parameter between subjects with and without recurrence assuming that ~50% of subjects will have developed recurrence. In this study the proposed 30 subjects also will allow capture of a significant Spearman ρ correlation as low as $\rho=0.50$ between ^{18}F -FLT and Ki67 index with 85% statistical power.

Logistic regression analysis will be performed to determine the ability of ^{18}F -FLT-PET for predicting tumor recurrence as a binary endpoint using each ^{18}F -FLT imaging parameter by univariate analysis.[39] Odds ratios and 95% confidence intervals (CI) will be computed as measures of diagnostic performance of ^{18}F -FLT lesion uptake with biopsy findings as the gold standard. Receiver-operating characteristic (ROC) curve analysis will be applied to assess area under the curve (A_z) as determined by the trapezoidal method and the optimal cutoff values for the various ^{18}F -FLT parameters determined by the Youden index.[40] Sensitivity and specificity with 95% confidence intervals will be calculated based on chosen cutoffs for each ^{18}F -FLT parameter. We anticipate enrolling ~10 patients per year and expect that the tumor recurrence rate will be approximately 50% at final assessment.

13.3 Study 3:

Children with recently diagnosed neuroepithelial central nervous system tumors, use ^{18}F -FLT-PET to characterize the tumor response to chemotherapy and evaluate if early tumor response to chemotherapy predicts clinical outcome.

This exploratory study will assess different ^{18}F -FLT parameters for characterizing tumor response as a predictor of clinical outcome.

Subjects will undergo ^{18}F -FLT-PET of the brain and/or spinal column at 2 time points: prior to initiation of new chemotherapy and after completion of the second cycle of chemotherapy (typically <2 months after start of chemotherapy). A subject will be categorized as a chemotherapy responder if the post-therapy scan shows

that tumor uptake has decreased to less than 75% of the uptake level seen on the pre-therapy scan. A subject will be categorized as a non-responder if the post-therapy uptake remains greater than 75% of pre-therapy uptake. Survival curves for these two groups (responders and non-responders) will be compared using the log-rank test, [41, 42] with an outcome event defined as progression or death during two years of clinical follow-up after the start of chemotherapy. In a group of children and young adults with heterogeneous tumor types, we estimate an event rate of 90% in non-responders and an event rate of 40% in FLT responders. Statistical analysis will be performed using SPSS software (version 17.0, SPSS Inc., Chicago, IL). Two-tailed $p < 0.05$ will be considered statistically significant.

Power calculations were performed using nQuery Advisor (version 7.0, Statistical Solutions, Saugus, MA), assuming that progression-free survival will be observed in 10% of non-responders and 60% of responders, with a hazard ratio of 4.508. To have an 80% chance of detecting a significant ($p \leq 0.05$) difference in the rate of progression-free response between these two groups, a sample size of 15 per group, or total enrollment of 30 will be needed. A minimum of 14 events (progression or death) will be required to detect a difference between the two groups.[43]

13.4 Assignment of subject numbers:

The anticipated number of new subjects recruited each year is outlined below. Subjects in Study 1 and Study 2 will have one ^{18}F -FLT-PET scan. Subjects in Study 3 will have two ^{18}F -FLT-PET scans (before and after chemotherapy). During year 4, no new subjects will be recruited to study 3 to permit a longer period of follow-up of patient outcomes during the period of the protocol. It is anticipated that no subjects would be recruited to Study 2 during the first year, to balance utilization of resources.

SUBJECT NUMBERS	Year 1	Year 2	Year 3	Year 4	Total
Study 1 (new diagnosis)	5	5	5	5	20
Study 2 (possible recurrence)	0	14	14	2	30
Study 3 (response to therapy)	10	10	10	0	30
Total	15	29	29	7	80

14 Publication of Findings

This clinical trial has been registered in the Protocol Registration System at ClinicalTrials.gov (NCT01244737). A good faith effort will be made to have a description of the findings of this study published in a peer-reviewed medical journal.

15 References

1. *Statistical Report: Primary Brain Tumors in the United States, 2000–2004.* , 2008, Central Brain Tumor Registry of the United States Hinsdale, IL. website: www.cbtrus.org.
2. Louis, D.N., et al., *The 2007 WHO classification of tumours of the central nervous system.* Acta Neuropathol, 2007. **114**(2): p. 97-109.
3. Wolfsberger, S., et al., *Ki-67 immunolabeling index is an accurate predictor of outcome in patients with intracranial ependymoma.* The American journal of surgical pathology, 2004. **28**(7): p. 914.
4. Preusser, M., et al., *Ki67 index in intracranial ependymoma: a promising histopathological candidate biomarker.* Histopathology, 2008. **53**(1): p. 39-47.
5. Rhee, W., *Quantitative analysis of mitotic Olig2 cells in adult human brain and gliomas: Implications for glioma histogenesis and biology.* Glia, 2008.
6. Shields, A.F., Graham, M.M., Spence, A.M., *The role of PET imaging in clinical oncology: A current status report.* Nuclear Med Annual, 1995: p. 129-168.
7. Herholz, K., J. Rudolf, and W.D. Heiss, *FDG transport and phosphorylation in human gliomas measured with dynamic PET.* J Neurooncol, 1992. **12**(2): p. 159-65.
8. Finlay, J.L. and S. Zacharoulis, *The treatment of high grade gliomas and diffuse intrinsic pontine tumors of childhood and adolescence: a historical - and futuristic - perspective.* J Neurooncol, 2005. **75**(3): p. 253-66.
9. Shields, A.F., et al., *Carbon-11-thymidine and FDG to measure therapy response.* J Nucl Med, 1998. **39**(10): p. 1757-62.
10. Romer, W., et al., *Positron emission tomography in non-Hodgkin's lymphoma: assessment of chemotherapy with fluorodeoxyglucose.* Blood, 1998. **91**(12): p. 4464-71.
11. Spence, A.M., D.A. Mankoff, and M. Muzi, *Positron emission tomography imaging of brain tumors.* Neuroimaging Clin N Am, 2003. **13**(4): p. 717-39.
12. Wong, T.Z., G.J. van der Westhuizen, and R.E. Coleman, *Positron emission tomography imaging of brain tumors.* Neuroimaging Clin N Am, 2002. **12**(4): p. 615-26.
13. Shreve, P.D., Y. Anzai, and R.L. Wahl, *Pitfalls in oncologic diagnosis with FDG PET imaging: physiologic and benign variants.* Radiographics, 1999. **19**(1): p. 61-77; quiz 150-1.
14. Kubota, R., et al., *Intratumoral distribution of fluorine-18-fluorodeoxyglucose in vivo: high accumulation in macrophages and granulation tissues studied by microautoradiography.* J Nucl Med, 1992. **33**(11): p. 1972-80.
15. Yamada, Y., et al., *Fluorine-18-fluorodeoxyglucose and carbon-11-methionine evaluation of lymphadenopathy in sarcoidosis.* J Nucl Med, 1998. **39**(7): p. 1160-6.
16. Arner, E.S. and S. Eriksson, *Mammalian deoxyribonucleoside kinases.* Pharmacol Ther, 1995. **67**(2): p. 155-86.
17. Sherley, J.L. and T.J. Kelly, *Regulation of human thymidine kinase during the cell cycle.* J Biol Chem, 1988. **263**(17): p. 8350-8.
18. Kong, X.B., et al., *Comparisons of anti-human immunodeficiency virus activities, cellular transport, and plasma and intracellular pharmacokinetics of 3'-fluoro-3'-deoxythymidine and 3'-azido-3'-deoxythymidine.* Antimicrob Agents Chemother, 1992. **36**(4): p. 808-18.
19. van Eijkeren, M.E., et al., *Kinetics of [methyl-11C]thymidine in patients with squamous cell carcinoma of the head and neck.* Acta Oncol, 1996. **35**(6): p. 737-41.
20. Vander Borgh, T., et al., *Production of [2-11C]thymidine for quantification of cellular proliferation with PET.* Int J Rad Appl Instrum A, 1991. **42**(1): p. 103-4.

21. Conti, P.S., et al., *Synthesis of 2'-fluoro-5-[11C]-methyl-1-beta-D-arabinofuranosyluracil ([11C]-FMAU): a potential nucleoside analog for in vivo study of cellular proliferation with PET*. Nucl Med Biol, 1995. **22**(6): p. 783-9.
22. Grierson, J., Shields, A.F., Courter, J.H., *Radiosynthesis of 2'-deoxy-1-[C-11]methyl-pseudouridine, an Isostere of labeled thymidine, as an agent for measurement of DNA synthesis in vivo*. J Labelled Compounds Radiopharm, 1994. **35**: p. 290-291.
23. Shields, A.F., et al., *Development of labeled thymidine analogs for imaging tumor proliferation*. Nucl Med Biol, 1996. **23**(1): p. 17-22.
24. Flexner, C., et al., *Relationship between Plasma-Concentrations of 3'-Deoxy-3'-Fluorothymidine (Alovudine) and Antiretroviral Activity in 2 Concentration-Controlled Trials*. Journal of Infectious Diseases, 1994. **170**(6): p. 1394-1403.
25. Dittmann, H., et al., *Early changes in [F-18]FLT uptake after chemotherapy: an experimental study*. European Journal of Nuclear Medicine and Molecular Imaging, 2002. **29**(11): p. 1462-1469.
26. Shields, A.F., et al., *Imaging proliferation in vivo with [F-18]FLT and positron emission tomography*. Nat Med, 1998. **4**(11): p. 1334-6.
27. Vesselle, H., et al., *In vivo validation of 3'-deoxy-3'-[(18)F]fluorothymidine ([18F]FLT) as a proliferation imaging tracer in humans: correlation of [18F]FLT uptake by positron emission tomography with Ki-67 immunohistochemistry and flow cytometry in human lung tumors*. Clin Cancer Res, 2002. **8**(11): p. 3315-23.
28. Buck, A.K., et al., *3-deoxy-3-[(18)F]fluorothymidine-positron emission tomography for noninvasive assessment of proliferation in pulmonary nodules*. Cancer Res, 2002. **62**(12): p. 3331-4.
29. Shields, A.F., *PET imaging with 18F-FLT and thymidine analogs: promise and pitfalls*. J Nucl Med, 2003. **44**(9): p. 1432-4.
30. Spence, A.M., et. al., *Initial assessment of [18F]3'-deoxy-3'-fluorothymidine (FLT) for PET imaging of DNA biosynthesis in glioma patients*. in 51st Annual Meeting of the Society of Nuclear Medicine. 2004. Philadelphia, PA.
31. Choi, S.J., et al., *[18F]3'-deoxy-3'-fluorothymidine PET for the diagnosis and grading of brain tumors*. Eur J Nucl Med Mol Imaging, 2005. **32**(6): p. 653-9.
32. Kim, S.e.a. *Direct comparison of F-18 FLT and F-18 FDG PET in patients with brain tumors*. in 51st Annual Meeting of the Society of Nuclear Medicine. 2004. Philadelphia, PA.
33. Chen, W., et al., *Imaging proliferation in brain tumors with 18F-FLT PET: comparison with 18F-FDG*. J Nucl Med, 2005. **46**(6): p. 945-52.
34. Fahey, F.H., et al., *Variability in PET quantitation within a multicenter consortium*. Med Phys, 2010. **37**(7): p. 3660-6.
35. Stabin, M.G. and J.A. Siegel, *Physical models and dose factors for use in internal dose assessment*. Health Physics, 2003. **85**(3): p. 294-310.
36. Vesselle, H., et.al., *18F-Fluorothymidine radiation dosimetry in human PET imaging studies*. J Nucl Med, 2003. **44**: p. 1482-8.
37. ICRP, 1998, *Radiation Dose to Patients from Radiopharmaceuticals (Addendum to ICRP Publication 53)*. ICRP Publication 80 1998; 28 (3):[Available from: <http://www.icrp.org/publication.asp?id=ICRP%20Publication%2080>].
38. Calvez, V., et al., *MIV-310 reduces markedly viral load in patients with virological failure despite multiple-drug therapy: results from a 4 week phase II study*. Antiviral Therapy, 2002. **7**: p. S4.
39. Hosmer, D. and S. Lemeshow, *Applied Logistic Regression.*, 2nd edn.(Wiley: New York.), 2000, CrossRef. p. 143-75.

40. Hanley, J.A., McNeil, BJ, *The meaning and use of the area under a receiver operating characteristic (ROC) curve*. Radiology, 1982. **143**: p. 29-36.
41. Kaplan, E.L. and P. Meier, *Nonparametric estimation from incomplete observations*. Journal of the American statistical association, 1958. **53**(282): p. 457-481.
42. Mantel, N., *Evaluation of survival data and two new rank order statistics arising in its consideration*. Cancer Chemother Rep, 1966. **50**(3): p. 163-70.
43. Freedman, L., *Tables of the number of patients required in clinical trials using logrank test*. Stat Med, 1982. **1**(2): p. 121-129.

Appendix A

Performance Scale

Karnofsky Performance Scale

- 100** Normal; no complaints; no evidence of disease.
- 90** Able to carry on normal activity; minor signs or symptoms of disease
- 80** Normal activity with effort; some signs or symptoms of disease
- 70** Cares for self; unable to carry on normal activity or do active work.
- 60** Requires occasional assistance, but is able to care for most of own needs.
- 50** Requires considerable assistance and frequent medical care.
- 40** Disabled; requires special care and assistance.
- 30** Severely disabled; hospitalization is indicated, although death is not imminent.
- 20** Hospitalization necessary; very sick; active supportive treatment necessary.
- 10** Moribund; fatal processes progressing rapidly.

Lansky Play-Performance Scale

- 100** Fully active, normal
- 90** Minor restrictions in physically strenuous activity.
- 80** Active, but tires more quickly.
- 70** Both greater restriction of and less time spent in play activity.
- 60** Up and around, but active play; keeps busy with quieter activities.
- 50** Gets dressed, but lies around much of the day, no active play, able to participate in all quiet play and activities.
- 40** Mostly in bed; participates in quiet activities.
- 30** In bed; needs assistance even for quiet activities.
- 20** Often sleeping; play entirely limited to very passive activities.
- 10** No play; does not get out of bed.
- 0** Unresponsive.