Memorial Sloan-Kettering Cancer Center
IRB Protocol

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Image-Guided Stereotactic Biopsy of High Grade Gliomas

MSKCC NON-THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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1.1 PROTOCOL SUMMARY AND/OR SCHEMA

This is a baseline feasibility study using fluorine-18-labeled fluorothymidine (^{18}F-FLT), a proliferation tracer, and highly focal MR image-guided biopsy to evaluate whether characteristic metabolic/MR imaging findings can be linked to distinct molecular phenotypes and improve our understanding of the molecular and pathological heterogeneity within individual tumors. In addition, this study will assess the feasibility of voxel-wise estimation of proliferation and tracer delivery from overall FLT uptake assessments, as against traditional ROI-based pharmacokinetic approaches. The quantitative accuracy of these parametric images will be investigated through comparison of histologic determinations of tumor cell proliferation, CD31 expression, and gene expression differences, obtained at different biopsy locations, with the PET-MRI imaging features at those same locations. The results of these studies will lay the groundwork for larger prospective studies assessing ^{18}F-FLT proliferative response in trials of targeted therapy, relating estimates of proliferation with prognosis, survival, and clinical outcome.

Please note that all patients entered on this protocol will undergo the standard of care for their brain tumor. The information obtained from the use of ^{18}F-FLT PET scans will NOT be used to guide patients' surgical planning on this protocol. Since FLT PET is considered investigational, subjects enrolled in this protocol will only undergo FLT PET scans under an FDA-approved institutional IND (#104742).

Schema:

a. Evaluation and consent of 15 patients with initial presentation of presumptive glioblastoma based on MRI appearance.
b. Baseline pretreatment MRI studies following injection of gadolinium (GdDTPA) and pre-surgical dynamic ^{18}F-FLT PET study with CT for attenuation correction.
c. Planned tumor resection volume will be pre-determined by the operating neurosurgeon using anatomic MRI per routine, who will be blinded to the results of the preoperative PET study.
d. Two neuroradiologists and a nuclear medicine attending, blinded to the surgical planning, will independently select "patterns of interest" or sites of heterogeneity within the tumor on co-registered PET-MRI scans for targeted biopsy.
e. Surgical resection will take place per routine with frameless stereotactic tracking of resection location, updated by intraoperative MRI (iMRI,1.5T Siemens magnet).
f. Histopathological and molecular characterization of the biopsied tumors: immunohistochemical staining for MIB-1 and gene expression profiling.
g. Data analysis: physician interpretation of brain MRI and PET scans; volumetric region-of-interest (ROI) analysis for derivation of tumor and contralateral brain activity; ^{18}F-FLT time activity curves (TACs) and steady-state SUVs; non-invasive determination of plasma TACs (venous and arterial input curves); voxel-based and ROI-based pharmacokinetic modeling.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary Aim: To investigate relationships between voxel-based determinations of proliferation rate and observed MR imaging features (i.e., K_{trans} or microvascular permeability; fBV, tissue fractional blood volume), as well as with spatially registered histologic measures of tumor cell proliferation (Ki67) and microvascular density (CD31) at corresponding locations.

Secondary Aim #1: To attempt to corroborate voxel-based parameter estimates reflecting tumor cell proliferation with estimates derived using standard ROI-based pharmacokinetic modeling methods, for improving the characterization of high-grade gliomas using dynamic ^{18}F-FLT PET-CT.

Secondary Aim #2: To assess whether static measures of ^{18}F-FLT uptake can adequately serve as non-invasive biomarkers of proliferative activity or whether parametric images, based on compartmental analyses of the FLT pharmacokinetics, are required by correlating findings of both approaches with regional histologic assays of tumor cell proliferation.

Exploratory Aim: To evaluate whether differences in gene expression seen between areas of increased and decreased proliferative activity on parametric maps define consistent differential transcriptome signatures for comparison with known molecular subclasses of GBM and known pathways.

3.1 BACKGROUND AND RATIONALE

3.2 Introduction

3'-deoxy-3'-^{18}F-fluorothymidine (FLT) is a PET radiotracer used to assess tumor cell proliferation[1]. Tumor-cell proliferation has been identified as an important surrogate marker of survival in brain tumor patients, as determined by staining of *ex vivo* biopsy specimens with proliferative markers such as Ki-67[2,3]. Imaging with FLT takes advantage of the fact that pyrimidine nucleosides and several of their analogues are phosphorylated to the respective monophosphate (MP) by thymidine kinase 1 (TK-1) and are incorporated into DNA. FLT is also a substrate for TK-1, which has been demonstrated both *in vitro*[4] and *in vivo*[5,6]. TK-1 activity is up-regulated in cells entering the S-phase, whereas the protein is nearly undetectable in growth arrested cells. TK-1 catalyzes the phosphorylation of FLT to FLT-monophosphate. Because it lacks a 3'-hydroxyl group, very little FLT is incorporated into DNA. Thus, in the amounts used for a radiotracer, it has no pharmacological properties and measures an early event in DNA synthesis rather than DNA incorporation. Intracellularly, phosphorylated FLT remains trapped. Nevertheless, many reports have shown a positive correlation between FLT uptake and S-phase fraction of cells *in* vitro and *in vivo*[7]. Its limited transport across the intact blood-brain barrier and the absence of proliferating cells in the normal adult brain result in low levels of ^{18}F-FLT accumulation within the brain. Accordingly, FLT-PET has been used to assess tumor proliferative status in high-grade glioma patients using static tracer uptake estimates and/or assessing time-dependent kinetic changes in uptake following intravenous administration of ^{18}F-FLT[7-18].

FLT has been synthesized by the MSKCC cyclotron facility for more than 2 years, and has been used in humans here and in several institutions in this country and Europe. For the purpose of this protocol, the synthesis will be done according to practice guidelines set forth in the NCI IND file. The primary source of the FLT will be the MSKCC cyclotron facility; however, alternative commercial

sources are available to ensure consistent supply of FLT. If a commercial supplier of FLT is used for a particular study, the supplier will be required to meet all of the acceptance criteria outlined in the FDA-approved MSKCC FLT IND (#104742) prior to administration.

3.3 ^{18}F-FLT Tracer Kinetic Modeling:

The utility of static (SUV) versus dynamic measures (i.e., k_3) of ^{18}F-FLT PET as an imaging biomarker represents different schools of thought, with examples of both found in the literature, and without a clear consensus offered[7,19,20]. Static measures of brain tumor ^{18}F-FLT uptake at various times post-injection have been shown to correlate with histopathologic proliferation markers (Ki-67) in patient studies[9,19]. More recently, measured FLT SUV values were used to assess treatment response in recurrent malignant gliomas[18]. However, it is not clear what the FLT SUV measurements in such studies reflect, as both changes in tracer delivery and/or metabolic trapping will contribute to net tracer uptake. As an alternative approach, several previous clinical investigations have assessed uptake in brain tumors dynamically, generally using ROIs having maximum proliferative activity, in conjunction with two-tissue compartment (four-rate constant) models[7,8,10,16], in order to estimate kinetic rate constants reflecting tracer transport and cellular proliferation. Both methods continue to be utilized for in vivo brain tumor applications, noting that recently recommended ^{18}F-FLT data acquisition and analysis techniques have included static PET imaging at 40-60 min and corresponding SUV measurements of tumor activity concentration for brain tumors[20].

Accurate assessment of proliferation in brain by ^{18}F-FLT PET requires analysis of uptake kinetics to extract separate rate constants for the initial transport (K_1) and subsequent metabolic (trapping or phosphorylation) phases of ^{18}F-FLT uptake (k_3), the latter rate constant being directly proportional to the number and/or rate of proliferating cells within the tumor volume. This pharmacokinetic tracer uptake analysis requires knowledge of the fraction of ^{18}F-FLT in the blood, given known variations in in-vivo blood clearance. In addition to direct arterial or arterialized venous blood sampling[21], a number of alternative methods have been utilized[22-28]. Non-invasive ROI assessments of blood pools identified on PET images have also been successfully performed using the internal carotid arteries[29-31] and venous sinuses[32,33]. However, the small size of these vessels (relative to the spatial resolution of PET tomographs) will require corrections for partial volume effects and spillover. The use of a venous, rather than an arterial, input function assumes that the tracer has a very low permeability, with no measurable extraction occurring in a first pass through the vascular bed[34]. Under these conditions, tracer concentrations in the large venous structures will be good approximations for those in the cerebral capillaries.

3.4 Voxel-wise Pharmacokinetic Modeling of Dynamic ^{18}F-FLT-PET Data:

Pharmacokinetic modeling software for performing voxel-wise parameter estimates across the entire tumor volume has recently become available for multi-compartmental kinetic analyses. BioGuide™ is a research software module that operates from within the Philips radiotherapy planning Pinnacle™ software. BioGuide™ includes a module that performs voxel-based pharmacokinetic modeling of dynamic PET image data as well as co-registered serial PET images. This kinetic modeling algorithm will compute voxel-wise parameter estimates of the compartmental model parameters (K_1, k_2, k_3, and k_4) defining tracer exchange between the blood and tumor tissue compartments, as well as V_b, the intravascular blood volume fraction, which will be estimated as a fifth parameter.

Voxulus, a Bio-Guide™ software program, is currently being used by the Department of Medical Physics for pharmacokinetic modeling of dynamically-acquired radiotracer (^{18}F-FMiso) uptake on a voxel-by-voxel basis in both experimental models and head and neck cancer patients. BioGuide™ will be applied to brain ^{18}F-FLT images using defined volume-of-interest measurements within the tumor and contralateral brain at the same location for generating voxel-wise parameter estimates. Parametric maps will then be determined using the estimated proliferation rate constant (k_3) values that, for kinetic FLT PET data, are expected to reflect the spatial distribution of rates of cellular proliferation.

3.5 Multimodal Image-Guided Surgical Targeting:

With the exception of one recent investigation in which multiple targeted biopsy specimens were retrieved along a single biopsy tract[17], image-based measurements of ^{18}F-FLT uptake have not been made in the region of histologic sampling. Given the high degree of heterogeneity of glial tumors, it would be preferable to correlate several imaging measurements per patient with histologic analyses in the same areas of the tumor in order to detail relationships between tumor metabolism, physiology, and specific malignant features. Local relationships between histologic features and radiotracer uptake or anatomic/functional MR imaging findings will additionally influence image analysis and interpretation of gliomas. Multimodal image-guided stereotactic biopsies have been previously performed by co-registering functional MR and PET imaging studies[17,35-37]. By combining anatomic scans with metabolic and functional data from PET imaging, complementary information may be obtained that permits tumor heterogeneity and extension to be better defined, in addition to adding prognostic value[37]. These studies have sought to detail relationships between functional imaging parameters reflecting tumor vascularity (relative microvascular blood volume, rCBV) and tumor metabolism using ^{11}C-methionine or ^{18}F-FDG, and their relationship to malignant features, MVD, or VEGF by histopathologic correlation. However, these correlations generally relied on representative tissue samples obtained from biopsy or resection, not in the region of image-based measurements[35,37]. Thus, local comparisons between imaging and tissue measurements could not be performed. Correlations between brain tumor ^{18}F-FLT uptake and histopathologic proliferation markers have also been based on representative biopsy or resection specimens[9]. Neuronavigated surgery, however, facilitates increased precision of tissue sampling at locations that correspond with imaging measurements.

3.6 Rationale

Improved molecular imaging of brain tumors has been linked to several research priorities, identified in the report published by the NCI/NINDS Brain Tumor Progress Review Group (November 2000), including (1) a need for establishing noninvasive markers of early treatment response and (2) the need to understand treatment-relevant molecular alterations in relation to anatomic site and tumor heterogeneity. There has been limited advancement on both fronts, in large part due to the difficulty of systematically acquiring targeted biopsies and correlating imaging with tissue measures. The clinical relevance of this proposal is the demonstration of feasibility for (1) highly focal image-guided biopsy that can power a prospective study of FLT as a marker of prognosis or early response (2) identifying molecular correlates of imaging heterogeneity.

Although a number of clinical FLT-PET investigations have assessed uptake in newly diagnosed or recurrent high-grade gliomas, using either static measures or dynamic approaches to estimate tracer kinetic parameters, these studies have not established FLT-PET as a prognostic or predictive marker.

This baseline study will serve to establish feasibility of performing analyses of prognostication post-hoc using the derived SUVs and/or rate constants reflecting proliferation/metabolic flux. If prognostication can be established, a larger patient cohort will be followed prospectively to assess clinical outcomes over longer time intervals. While baseline FLT-PET studies have not offered prognostic information, changes in FLT SUV values during treatment have been investigated as a predictor of long-term outcome. It is important to determine the component of FLT uptake measurements that may correlate with tumor cell proliferation (Ki-67), which is intriguing as a potential biomarker, given that it was predictive of overall survival in bevacizumab- and irinotecan-treated recurrent gliomas as early as 1-2 wk after treatment. By establishing such response predictors and refining our ability to detect response to novel therapies in malignant brain tumor clinical trials, treatment protocols can be more efficiently optimized, expediting the evaluation of promising compounds in clinical trials for assessing patient outcomes.

Tailoring targeted therapies based on an individual tumor's unique molecular and biochemical signatures is an ultimate goal in managing brain tumor patients. Characterizing tumors at multiple sites, guided by heterogeneous metabolic/functional imaging findings, may capture variation in potential tissue biomarkers, such as EGFR amplification or MGMT methylation, which are frequently heterogeneously distributed. To meet this challenge, it will be necessary to determine clinically useful biomarkers with prognostic and predictive value to improve selection of specific therapeutics and to assess early response/progression for modifying treatment protocols. High throughput molecular analyses of surgically procured tissue have enhanced the characterization of gliomas, resulting in the definition of novel tumor sub-classes, better diagnostic accuracy, and improved prognostication. Prolonged progression-free and overall survival in glioblastoma patients treated with DNA alkylating agents, for instance, has been linked to MGMT hypermethylation in the majority of tumors. The results of such studies have suggested that gene expression profiling will impact future clinical practice in terms of cancer diagnosis, prognosis, and choice of optimal treatment. Under certain conditions, however, such as during the course of treatment, brain tumor biopsy may be impractical, and given that radiographic disease progression is often associated with morbid decline, there is a critical need to establish early and reliable non-invasive biomarkers of treatment response.

This study will also lay the groundwork for establishing the interpretability of ^{18}F-FLT heterogeneity within and across tumors on a voxel-wise basis. The feasibility of voxel-wise estimation of proliferation will be established, and validated against traditional ROI-based pharmacokinetic approaches. The results of these studies may inform the design of larger prospective studies assessing ^{18}F-FLT proliferative response in trial of targeted therapies, as well as determine the potential for FLT-PET to serve as a biomarker for proliferation at the tissue level. An FDA-approved IND has been issued for ^{18}F-FLT (#104742) at this institution.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is a two-year open-label pilot study at Memorial Sloan Kettering Cancer Center that will enroll 15 patients with initial presentation of presumptive glioblastoma based on prior radiographic studies for ^{18}F-FLT PET imaging correlation with histologic and molecular markers. Standard baseline pretreatment MRI studies will be obtained following injection of gadolinium (GdDTPA) and a pre-surgical dynamic ^{18}F-FLT PET study with CT for attenuation correction will be performed on Day 1. Surgical resection will be performed within a 7 day period of these imaging studies. The planned tumor resection volume will be pre-determined by the operating neurosurgeon on the basis of anatomic/functional MRI per routine, and blinded to the results of the preoperative PET study. Two neuroradiologists (Drs. Michelle Bradbury and Sasan Karimi) and a nuclear medicine attending (Dr. Heiko Schöder), blinded to the surgical planning, will independently identify “patterns of interest” or sites of heterogeneity within the tumor on co-registered PET-MRI scans for targeted biopsy. Fused images containing the marked biopsy sites will be uploaded to the BrainLAB VectorVision® neuronavigation system for viewing by the neurosurgeon in the operating room. Selected targeted sites will be marked as potential biopsy targets if they are: (1) contained with the pre-determined planned tumor resection volume, (2) over 1cm^3, and (3) abnormal on intraoperative visualization. Surgical resection will take place per routine with frameless stereotactic tracking of resection location, updated by intraoperative MRI (iMRI, 1.5T Siemens magnet). As target sites are encountered, up to 1gm of tumor will be marked as a separate biopsy specimen and flash frozen in liquid nitrogen. Biopsy specimens will be taken only after adequate material has been acquired for routine pathology. Prior to histopathologic and molecular characterization, biopsy specimens will be inspected by the pathologist to insure adequacy of tumor tissue sampling and to definitively diagnose tumor. Histopathologic and molecular characterization of the biopsied tumors will be performed, specifically immunohistochemical staining for MIB-1, in addition to gene expression profiling. Data analysis will include physician interpretation of brain MRI and PET scans, volumetric region-of-interest (ROI) analyses of tumor and contralateral brain, generation of ^{18}F-FLT time activity curves (TACs) and steady-state SUVs, non-invasive determination of plasma TACs (venous and arterial input curves), as well as voxel-based and ROI-based pharmacokinetic modeling. *Periodic meetings will be held to discuss issues related to data acquisition, collection, and analysis, as well as to discuss the imaging and histopathology data.*

Primary Aim Study Design: Preoperative anatomical MRI images will be uploaded to the BrainLAB VectorVision® neuronavigation system, a frameless stereotaxy and image-guided surgical navigation system integrated with the Intraoperative MRI suite. Planned resection volume will be contoured by the neurosurgeon conventionally, based on the anatomic MRI. ^{18}F-FLT PET-MRI studies will then be uploaded and registered by a radiologist. For each patient undergoing resection, up to four prescribed ROIs within the planned resection volume will be selected for potential targeted biopsy based on concordance/discordance or heterogeneity of PET and MRI imaging characteristics (i.e., increased ^{18}F-FLT uptake/MRI-enhancing; increased ^{18}F-FLT uptake/MRI-non-enhancing; low ^{18}F-FLT uptake/MRI-enhancing; increased ^{18}F-FLT uptake/MRI-nonenhancing). ROIs will be no smaller than a cubic centimeter. The operative plan, in terms of the tumor resection volume, will not be guided by decisions regarding sites of potential biopsy selected on the co-registered FLT PET-MRI study. It is expected that

the majority of target sites selected for each patient (up to 4) will be confined within the area determined for resection; these tumors are usually large at presentation with heterogeneous enhancement and rim enhancement. Thus, it is expected that several areas selected by the neuroradiologists and nuclear medicine attending will be biopsied. Frameless stereotaxy will be utilized to estimate proximity to an ROI during resection. An intraoperative MRI will be obtained when one or more ROIs are accessible for biopsy. Multiple biopsies will then be acquired from the vicinity of the ROI with recording of the stereotactic locations of the sites and collection in separately numbered tubes.

Brain shift and deformation will likely be present at the time of biopsy, shown by intraoperative MRI performed immediately before or after biopsy, limiting our ability to determine whether or not an ROI has been accurately sampled until after the procedure. Non-linear registration algorithms will be applied to correct for geometric distortions, and will be applied after maximizing global imaging registration using a linear registration algorithm. Non-linear registration methods will permit us to further adjust and refine post-hoc accuracy of tumor tissue sampling with regard to the preselected target ROIs, thereby enabling biopsied samples to be accepted or rejected. We have chosen to perform this compensation of brain shift post-hoc because it is procedurally time-consuming and might be subject to errors that could potentially misdirect the surgeon if used to directly guide biopsy. Instead, our method relies on the ability to take multiple biopsies from the vicinity of an ROI, annotating each with accurate localization based on intraoperative MRI. The accuracy of localization is about 1 cm^3. Such piecewise acquisition of tissue is a routine approach for obtaining pathology and banking specimens at our institution.

Post-hoc mapping of biopsy sites into presurgical image coordinates will determine which samples were in fact within the target ROI. The primary limitation of the approach is that it relies on the surgeon's estimation of where an ROI has shifted intraoperatively in order to direct biopsies: it is possible that some ROIs may be missed or deemed to be outside the resection volume and hence unavailable for biopsy. However, the minimum proposed ROI size of 1.0 cm^3 is within the range of intraoperative accuracy routinely used by neurosurgeons during resection to avoid vascular and neuroanatomic structures. Therefore it is anticipated that at least one specimen be taken from the vicinity of the ROI, as judged by the surgeon; it will be established post-hoc as being accurately mapped to within an ROI. Acquiring tissue from surgically exposed tumor, as proposed here, avoids the significant risk of parenchymal bleeding from deep needle biopsy. If difficulties are encountered implementing the above plan, needle biopsy sampling through an initial minimal surgical exposure will be employed under a separate IRB-approved protocol.

Biopsy specimens will be acquired for research purposes after pathologic specimens are submitted for the patient per routine by neurosurgery. A minimum of 1.0 g of biopsy tissue will be acquired per patient for research purposes, as the priority for the study is histologic validation. We will ensure that as much tissue as possible is sent for histology, aiming for roughly half of the specimens to be further processed as frozen sections for research purposes.

Biopsy specimens will be frozen immediately in liquid nitrogen and stored at -80 °C until later use. All biopsy cores will be divided in half, with one half of the frozen tissue specimen sectioned for RNA and DNA expression analysis, and the remaining half processed as a formalin-fixed paraffin-embedded (FFPE) specimen for immunohistochemistry by fixing the tissue in 10% neutral buffered formalin for 2 days, followed by paraffin embedding. Tissue markers for tumor cell proliferation (MIB-1) and

microvessel density (CD31) will be applied to FFPE sections. Serial sections will be reviewed by an experienced neuropathologist. Ki-67 staining will be evaluated and a score designated indicating the percentage of positively stained tumor cells per quartile of tumor tissue. All cells with nuclei staining of any intensity will be defined as positive. The proliferative activity score, quantified as the percentage of MIB-1 stained nuclei per total nuclei in the sample, will be estimated from a representative slide selected by the neuropathologist. For MVD assessment using CD31, tumor sections will be scanned at low magnification (x40) to identify the region of the section with the highest microvessel density. This area will be counted at a magnification of x200 for CD31-positive expression, and the proportion of CD31-positive cells computed for all specimens.

Histologic findings at a given biopsy site will be compared with ROI analyses at the same location on the co-registered PET-MRI studies. Pharmacokinetic modeling will be applied to these ROI measurements in order to generate parametric maps reflecting the proliferation rate constant, k_3, and K_{trans} (microvascular permeability). Estimated parameter values will be correlated with the presence of molecular markers (MIB-1, CD31), and compared with imaging features at the prospectively defined biopsy sites.

Secondary Aim #1 Study Design: The objective will be to investigate the feasibility of applying a novel imaging software platform to the measured dynamic brain PET imaging data. Voxel-based parameter estimates will be generated using ROIs selected for targeted biopsy and, in turn, utilized to construct parametric images (maps) reflecting the spatial distribution of the rate of ^{18}F-FLT cellular trapping (or proliferation). These estimates will be compared with kinetic parameters determined using traditional ROI-based pharmacokinetic modeling methods. As input to the model, venous time-activity curves will be derived non-invasively at the level of the confluence of the sagittal, straight, and transverse sinuses, and corrected for hematocrit and partial volume effects.

Venous input functions will be generated using previously described protocols[32]. We will use a summed image of the first 2 frames (1 min) of the study and place small ROIs (5 mm) on two to five successive planes in the image at the level of the confluence of the superior sagittal, transverse, and straight sinus, covering a total axial depth of 1.0-1.5 cm. The co-registered MRI and PET-CT will be used to confirm our interpretation of the vascular anatomy revealed by PET and to verify the position of the ROIs. In addition, threshold values of 50% of the peak activity in the sinuses will be used for further guiding the placement of the ROIs, with data for the ROIs on successive planes averaged. The resulting time course of the SUV will be automatically corrected for detection efficiency, scatter attenuation, and decay. In order to correct for partial volume averaging effects, the transverse dimension of the confluence of sinuses will be measured on axial T2-weighted imaging. Using the PET system recovery coefficient, derived from phantom studies as a function of the source dimension, a recovery coefficient for the confluence of sinuses will be determined. Each TAC data point will be divided by this recovery coefficient to yield the corrected TAC data.

Venous input functions will be compared with non-invasively derived arterial input functions, the latter determined from the internal carotid arteries at the level of the skull base on PET after fusing with MR images for artery identification. The feasibility of using arterial structures at this level will be investigated using the same method described above for determining venous input functions. The agreement of kinetic parameter values calculated using both venous and arterial input functions will be examined.

A number of studies have compared rate constants estimated using non-invasive, image-based input functions *versus* time-activity curves (TACs) generated from sampled arteries or arterialized veins, and have found no statistically significant differences for rate constants obtained with these input functions[32,39,40]. More recently, *image-derived* input functions for transverse and cavernous sinuses have been used in conjunction with compartmental modeling to investigate the kinetic behavior of *malignant brain tumors*, with the resulting rate constants found to adequately describe ^{18}F-FLT uptake characteristics[7]. Thus, as the collective results of these studies have demonstrated no statistically significant differences between blood samples and tomographically obtained input functions in terms of the kinetic parameters estimated, it is not essential that blood samples be acquired for determining an input function. However, up to 4 blood samples (approximately 3-milliliter per sample) may be taken post-injection, within the 1 hr imaging time period (i.e., roughly 15, 30, 45, and 60 min), for calibrating the corresponding image-based FLT TAC and/or improving pharmacokinetic parameter estimates, as well as for test consistency.). It should be noted that a range of post-injection time points (ultimately defining the blood TAC) can be selected for blood draws, as estimated pharmacokinetic parameters will not be critically dependent on the exact times chosen. This procedure will be performed in a number of patients to establish whether there is a relatively constant calibration factor or improved pharmacokinetic data that can be derived, which in turn would be applied to all the remaining patient data analyzed.

Secondary Aim #2 Study Design: Voxel-wise parameter estimates derived in secondary aim #1 will be compared with static measures of ^{18}F-FLT uptake using ROIs selected for targeted biopsy. To obtain static ^{18}F-FLT images, data will be summed between 30 and 60 minutes post-injection. Estimated parameter values will be correlated with the proliferative activity score or the percentage of MIB-1 stained nuclei per total nuclei in the sample as described under the Primary Aim Study Design.

Exploratory Aim Study Design: Biopsy samples will be acquired from regions of increased/decreased proliferative activity (FLT uptake). Gene expression from microdissected portions will be assayed by Affymetrix U133Plus2 arrays. Differential gene expression signatures will be derived for each pair of samples in a resection, representing differences between FLT uptake values. Signatures will be compared across samples to attempt to characterize a core signature. Core signatures will be investigated by comparison to known transcriptome signatures of molecular subclasses of GBM as well as compared with other pathways and signatures.

4.3 Intervention

PET scan protocol

- Fifteen (15) patients with newly diagnosed or recurrent gliomas, deemed appropriate for this scan by the investigator on the basis of imaging studies and surgical management considerations, will undergo the FLT-PET study for purposes of collecting pilot data (in compliance with RDRC regulations).

- All studies will be performed using a dedicated PET/CT, which integrates a dedicated PET scanner and a spiral CT with proprietary fusion software. Images are acquired with the patient in the fasted

state (about four to six hours), but water for hydration is allowed. A low dose spiral CT is performed first, followed by acquisition of PET images (emission images only), of the brain. Attenuation correction of the PET/CT images is performed using the CT data. PET, CT and fusion images are displayed on a standard workstation.

- A IV catheter will be placed in a superficial hand or arm vein for administration of ^{18}F-FLT (approximately 370 MBq), prepared by the MSKCC Radiochemistry Core Facility. A second venous catheter will be placed in the opposite hand or arm for venous blood sampling. If a central venous catheter is present, it will be used for blood sampling or radiopharmaceutical administration, and only a single venous catheter will be placed. Sequential blood samples may be obtained following ^{18}F-FLT infusion for assaying whole blood and plasma radioactivity. All catheters will be removed at the end of the day.

- Dynamic PET/CT images of the brain will be acquired for up to 1-hr on a dedicated PET/CT machine, with image acquisition starting immediately after injection using the following sequence: 6 frames of 30sec, 7 frames of 1-minute, then 10 frames of 5-minutes.

- The intravenous line will be removed at the end of the study.

- Venous blood samples (up to four) may be drawn from a vein in the arm opposite to that used to inject FLT by research staff. A single sample (approximately 3 milliliters) will be acquired at the end of the study (i.e., 60 min) for calibrating the FLT TAC, with the option to perform additional blood sampling (i.e., 15, 30, 45 min post-injection) for improving estimates of pharmacokinetic parameters. ^{18}F radioactivity in the ^{18}F-FLT blood/plasma samples will be measured by research staff.

- Dynamic FLT scans will be reconstructed by both filtered back projection and iterative reconstruction in order to check for quantitative accuracy at the smaller time segments immediately post injection.

- ^{18}F-FLT PET and CT examinations will be fused using a Vector-Vision® cranial navigation software (BrainLab AG, Heimstetten, Germany), after being transferred to a commercial workstation, BrainLab 1 (BrainLab AG).

- The ^{18}F-FLT PET-CT and baseline MRI studies will be uploaded onto BrainLab 1 and co-registered by the neuroradiologist using Vector-Vision® cranial navigation software. This will not be done in the operating room. The PET and MR image sets will be co-registered by applying the 3D rigid transformation (translations/rotations) to the PET image set, which aligns the CT image set of the PET-CT study and the MR image set.

- The operating neurosurgeon will be blinded to the pre-operative PET study, and planned tumor resection volume will be pre-determined solely on the basis of anatomic MRI per routine. Preoperative MRI images will be uploaded to the BrainLAB VectorVision® neuronavigation

system, a frameless stereotaxy and image-guided surgical navigation system integrated with the Intraoperative MRI suite.

- On co-registered PET-MRI scans and prior to surgical resection, up to four prescribed ROIs within the planned resection volume will be marked for potential targeted biopsy by two neuroradiologists and a nuclear medicine attending, blinded to the surgical planning. These ROIs will be selected based on heterogeneity of PET and MRI imaging characteristics (e.g., high ^{18}F-FLT uptake/no enhancement; low ^{18}F-FLT uptake/enhancement). Fused images containing the marked biopsy sites will be uploaded to the BrainLAB VectorVision® neuronavigation system for viewing by the neurosurgeon in the operating room. As noted above, however, the operative plan, in terms of the tumor resection volume, will not be guided by decisions regarding sites of potential biopsy selected on the co-registered FLT PET-MRI study.

- ROI analyses for determination of tracer uptake measurements and the generation of time-activity curves for blood and tumor will be performed on an AW Workstation (GE Healthcare) that runs the Voxulus™ software module for voxel-based pharmacokinetic modeling. Voxel-based pharmacokinetic modeling, traditional ROI-based pharmacokinetic modeling (*SAAM II* program), and static uptake determinations will all be performed on this workstation. The information derived from these analyses will not be used for surgical planning or uploaded for use in the operating room.

- Non-invasive input functions for blood will be obtained non-invasively using post-injection region-of-interest measurements of the superior sagittal sinus. These measurements will be compared with arterial blood activity-concentrations from an image containing the internal carotid artery, and will be calibrated using the measurement of a single intravenous blood sample acquired at one post-injection time point. If additional blood sampling (i.e., 15, 30, 45 min post-injection) has been acquired, these measurements will be compared with non-invasively derived values and used to improve estimates of pharmacokinetic parameters.

- Surgical resection and tissue specimen acquisition will take place with frameless stereotactic tracking of resection location and updated by intraoperative MRI.

- The radiation dose from the FLT studies was calculated based on published data. Given a realistic 1- hour voiding interval, the target organ is the liver, receiving a dose of 0.168 rad/mCi, corresponding to 1.68 rad/10mCi. This is below the 5 rad single organ permissible dose. Total body dose is calculated at 0.046 rad/mCi, and 0.46 rad for a 10 mCi injection, the maximum dose that will be used (see Table below). With a 1 hour voiding interval, the dose to the urinary bladder wall will be 0.146 rad/mCi. Recent CT dosimetry literature quotes a dose of 0.9 rad/single organ from a typical CT scan, performed for the purpose of PET attenuation correction on a PET-CT scanner. The diagnostic protocol described here will not exceed the permissible limit of 5 rad/organ/single study and will also remain below the maximal permissible dose acceptable to the radioactive drug research.

- **F18-Fluorothymidine (FLT) Patient Dosimetry**

Dosimetry for one PET FLT - CT scan

One FLT PET-CT scan	F18-FLT[2]		Absorbed Dose 1 FLT *PET-CT* scans 0.9 rad[3] + 10 mCi F18-FLT
Target Organ	**rad/mCi**	**rad/10 mCi**	**rad**
Adrenals	**0.077**	**0.766**	**1.67**
Bone Surfaces	**0.058**	**0.585**	**1.48**
Brain	**0.013**	**0.125**	**1.03**
Breasts	**0.031**	**0.310**	**1.21**
Heart Wall	**0.062**	**0.618**	**1.52**
Kidneys	**0.132**	**1.317**	**2.22**
Large Intestine - Lower Wall	**0.048**	**0.477**	**1.38**
Large Intestine - Upper Wall	**0.046**	**0.459**	**1.36**
Liver	**0.168**	**1.680**	**2.58**
Lungs	**0.037**	**0.374**	**1.27**
Pancreas	**0.085**	**0.851**	**1.75**
Red Marrow	**0.089**	**0.888**	**1.79**
Small Intestine	**0.053**	**0.525**	**1.43**
Stomach Wall	**0.052**	**0.522**	**1.42**
Testes	**0.049**	**0.488**	**1.39**
Thyroid	**0.038**	**0.385**	**1.28**
Urinary Bladder Wall			
45-min Voiding Interval	**0.110**	**1.098**	**2.00**
1-hr Voiding Interval	**0.146**	**1.463**	**2.36**
1.5-hr Voiding Interval	**0.220**	**2.195**	**3.10**
2-hr Voiding Interval	**0.293**	**2.927**	**3.83**

5.1 CRITERIA FOR SUBJECT ELIGIBILITY

The patient population will be comprised of patients with newly-diagnosed or recurrent high grade gliomas.

5.2 Subject Inclusion Criteria

5.1.1. Age $\geq$ 18 years old.

5.1.2. Radiographic appearance of a lesion presumed to be high-grade glioma.

5.1.3. Planned surgical resection.

5.2 Subject Exclusion Criteria

5.2.1. Pregnant (confirmed by serum b-HCG in women of reproductive age) or breast feeding

5.2.2 Patients with other active malignancies or prior treatment for non-CNS malignancies.

6.0 RECRUITMENT PLAN

Patients will be evaluated by attending physicians from the Departments of Neurosurgery and Radiology entered onto the study if they are appropriate candidates. The attending physician will obtain informed consent from the eligible patient.

7.0 ASSESSMENT/EVALUATION PLAN

Patient assessment and evaluation will be based on region-of-interest (ROI) measurements of tumor, non-tumor-bearing brain, the dural venous sinus/arterial blood pool, as well as additional data from whole blood/plasma radioactivity measurements. These assessments will be used to obtain static and dynamic ^{18}F-FLT uptake measurements, namely SUV values and estimated kinetic rate constants (including proliferation) using both voxel-wise and ROI-based tracer kinetic modeling approaches. SUV values and rate constants will be compared with molecular markers (percentage of Ki-67 and CD31 expressing cells, differential gene expression signatures) determined at corresponding sites of biopsy. This evaluation is detailed in Section 4.0.

8.0 TOXICITIES/SIDE EFFECTS

The radiotracer, ^{18}F-FLT, has been synthesized by the MSKCC cyclotron facility for about two years, and has been used in humans in this and other institutions in this country and Europe. The primary source of the FLT will be the MSKCC cyclotron facility; however, alternative commercial sources are available to ensure consistent supply of FLT. If a commercial supplier of FLT is used for a particular study, the supplier will be required to meet all of the acceptance criteria outlined in the FDA-approved MSKCC FLT IND (#104742) prior to administration. ^{18}F-FLT synthesized at MSKCC has been tested for toxicity and pyrogenicity. The product synthesized at MSKCC has met all requirements. No side effects are expected as a result of this study. However, in the unlikely event that an adverse reaction to the radiopharmaceutical occurs, the results must be documented and reported by the Principal Investigator to the Institutional Review Board Chairman and IND Committee.

9.0 PRIMARY OUTCOMES

Primary study measurements will be FLT uptake (SUV values); estimated rate constants, including that reflecting proliferation, derived from voxel-wise and ROI- based tracer kinetic modeling; and molecular markers (percentage of Ki-67 and CD31 expressing cells, differential gene expression signatures).

10.1 CRITERIA FOR REMOVAL FROM STUDY

10.1.1. Patients will not be removed from the protocol unless they request so.

10.1.2. If at any time, the patient develops unacceptable protocol-associated toxicity, he/she will be removed from study.

10.1.3. If at any time the patient is found to be ineligible for the protocol, as designated in the section on Criteria for Patient/Subject Eligibility (i.e., a change in diagnosis), the patient will be removed from the study.

11.0 BIOSTATISTICS

This is a pilot study intended to collect preliminary data on 15 patients diagnosed with untreated high-grade glioma who are scheduled to undergo surgical resection.

Primary Aim: To investigate relationships between voxel-based determinations of proliferation rate and observed MR imaging features, as well as with spatially registered histologic measures of tumor cell proliferation (Ki67) and microvascular density (CD31) at corresponding locations.

Statistical Methodology for Primary Aim: In this aim, we will first explore the relationships between ^{18}F-FLT PET parameters and MR imaging features (K_{trans} or microvascular permeability). The ^{18}F-FLT PET parameters: K_1 (tracer delivery rate constant), k_2 (rate constant reflecting flow from the tumor interstitial compartment back to blood), k_3 (rate constant reflecting proliferation), and k_4 (rate constant reflecting flow from tumor cells back into the tumor interstitial space) will be compared to K_{trans} on MR imaging. We will also explore relationships between K_{trans}, each of the ^{18}F-FLT PET parameters, and the histological markers Ki67 and CD31. All of the parameters and markers are measured on a continuous scale. Initially graphical methods such as pair-wise scatter plots will be used to assess the relationships. The relationships between each pair of variables will be more formally evaluated using methods that account for correlated data (there may be up to four observations per patient) such as random effects regression models or generalized estimating equations.

It is unlikely but possible that it may be determined at surgery that a patient does not have a high-grade glioma but instead has a metastasis or PCNSL. The likelihood that this will occur will be infrequent, if at all, given the constellation of distinctive imaging features that generally serve to differentiate these tumor types. If it does occur, however, we will continue accrual until 15 high grade glioma patients have been enrolled. In this case, two sets of analysis for the primary aim and all other aims will occur, one including all enrolled patients and one including only patients with high-grade gliomas. Patients found to have a metastasis or PCNSL may not yield different values than patients with glioma so the decision to include them in the analysis may not be unreasonable.

However, we will descriptively assess any differences in results between the two sets of analysis as much as the data permits if this situation arises.

Secondary Aim #1: To attempt to corroborate voxel-based parameter estimates reflecting tumor cell proliferation with estimates derived using standard ROI-based pharmacokinetic modeling methods, for improving the characterization of high-grade gliomas using dynamic ^{18}F-FLT PET-CT.

Statistical Methodology for Secondary Aim #1: To corroborate the voxel-based method with the ROI-based approach, pharmacokinetic modeling will be assessed by comparing the estimated parameter values for the entire intra-tumor ROIs to the average of the voxel-by-voxel parameter values among the voxels comprising the same ROIs. This analysis will focus on the k_3 value. For each ROI, we would like to see the estimated k_3 value derived from the entire ROI to be within 25% of the average k_3 value determined for the multiple voxels. The ratio of two k_3 values will be computed for this purpose. As an additional analysis, we will calculate the concordance correlation coefficient (CCC) to evaluate the agreement between the ROI-based and the average voxel-based parameter values. A modified version of the CCC that allows for clustered data will adjust for the fact that there may be multiple observations per patient contributing to the estimates[38].

Secondary Aim #2: To assess whether static measures of ^{18}F-FLT uptake can adequately serve as non-invasive biomarkers of proliferative activity or whether parametric images, based on compartmental analyses of the FLT pharmacokinetics, are required by correlating findings of both approaches with regional histologic assays of tumor cell proliferation.

Statistical Methodology for Secondary Aim #2: Using the same methods described for the primary arm, the relationship between Ki67 and the static measure of ^{18}F-FLT (the SUV), and the parametric measure (k_3) will be explored. The strength of the relationship between these two parameters and Ki67 will be compared descriptively.

Exploratory Aim: To evaluate whether differences in gene expression seen between areas of increased and decreased proliferative activity on parametric maps define consistent differential transcriptome signatures for comparison with known molecular subclasses of GBM and known pathways.

Statistical Methodology for Exploratory Aim:

ACGH: Array CGH data will be analyzed through the software pipeline developed within MSKCC's Cancer Genome Characterization Center within The Cancer Genome Atlas Project. This includes extensive QC annotation, normalization and artifact reduction, and segmentation analysis.

Expression analysis for gene signatures: Expression array data will be normalized by RMA and differential expression of paired samples determined by Significance Analysis of Microarrays (SAM). Methods of analysis cross-sample will aim at determining core signature genes which are commonly differentially expressed in relation to imaging features. These methods will depend on the

number of samples from each tumor that represent differential features. For larger sample sets, simple Venn intersection may reveal core signature genes. Sparser samplings of feature contrasts will be analyzed by pairwise Gene Set Enrichment to identify a subset of mutually expressed signature genes.

Analysis of gene signatures: Gene signatures representing contrasting imaging features will be compared with known signatures representing previously described molecular subclasses of GBM, including the "Proneural", "Proliferative" and "Mesenchymal" classes, as well as molecular subclasses already emergent in the analysis of 206 GBM samples in The Cancer Genome Atlas pilot project to date. Signatures will be compared by GSEA and gene ontology. GSEA will also be used to analyze feature-derived signatures in an exploratory process to characterize possible enrichment for altered expression within canonical pathways. The Connectivity Map offers another potentially valuable resource for mining feature-derived signatures, and can be approached with specific hypothesis: that differential proliferation and permeability, as image-derived features, may be associated with transcriptome evidence of PI3 kinase signaling or VEGF signaling, both of which are well represented in the database. Analysis will include exclusion of gene subsets such as cell cycle members whose differential regulation is likely to correlate with simple histologic measures of proliferation.

For the exploratory aim, we can evaluate the power we will have to detect a difference in gene expression levels between areas of high and low tracer uptake (or signal intensity) within an individual. With 15 subjects contributing a sample from both a high and low uptake area, we expect to be able to detect a twofold difference in base 2 log intensity levels with a Type I error of 0.001 and a Type II error of 0.05 if the standard deviation for the expression levels is 0.75 (using base 2 logarithms) or less. A standard deviation of 0.75 is within the range of what we expect to see[41].

<u>Sample size justification:</u>
For this pilot study, we expect there to be 15 eligible patients per year, and we will aim to reasonably accrue 8 of these patients per year for two years.

All analyses are purely exploratory and intended to generate data for future studies. We do not plan to conduct any rigorous hypothesis testing. To explore what we might be able to say using this limited sample size, we find that with data on 15 patients we expect to be able to detect a moderately high correlation coefficient of 0.60 with 80% power using a two-sided 0.05 level test[42]. This calculation assumes that each patient contributes only one observation, but in our case, we expect an average of two observations for each patient (up to four samples per patient) which may increase our power to draw any conclusions. Simulation studies assuming an average of two observations per patient indicate that we can estimate the concordance correlation coefficient to within (half-width of 95% confidence interval) ±0.27 for a CCC equal to 0.8, ±0.20 for a CCC equal to 0.9, and ±0.05 for a CCC equal to 0.99.

12.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

12.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. The PPR fax numbers are (646) 735-0008 and (646) 735-0003. Registrations can be phoned in or faxed. The completed signature page of the written consent/verbal script and a completed Eligibility Checklist must be faxed to PPR.

13.1 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinating the activities of the protocol study team.

The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record. All research material from this study will be handled with the same confidentiality as the patient's other medical data.

13.2 Quality Assurance

Eligibility of patients will be verified with the principal investigator. Only the designated investigators can obtain informed consent.

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for the level of risk and the degree of required monitoring. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) is reviewed and monitoring procedures are established at the time of protocol activation.

13.3 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at *http://cancertrials.nci.nih.gov/researchers/dsm/index.html*. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: *http://mskweb2.mskcc.org/irb/index.htm*.

14.1 PROTECTION OF HUMAN SUBJECTS

There are no foreseen additional risks to the patients from this study.

Risks of Study Participation: Patients in this study will be receiving current standard of care for their specific disease site.

Financial costs/burdens:
Because all diagnostic and therapeutic interventions except for the FLT-PET scans are part of the current routine care of patients/subjects eligible for this study and that a research grant will cover the cost of the FLT-PET scans, there are no additional financial cost or burden to the patient beyond the charges routinely incurred as part of standard medical care.

Patient Confidentiality:
Patient/subject privacy and confidentiality will be maintained according to MSKCC guidelines and all data derived from this study will be kept in a secure database. All data and results will be anonymously reported with regard to individual subjects.

Voluntary nature of the study:
Subjects will be made aware of the voluntary nature of the study as part of the informed consent process. They will be allowed to withdraw participation at any time without the risk of alteration in the quality of their medical care.

14.2 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board.

14.3 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org containing the following information:

Fields populated from the CRDB:
- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:
- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following information:
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

15.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form..

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