Serial [^{18}F]Fluorothymidine (FLT)PET/CT as a Biomarker of Therapeutic Response in Pemetrexed Therapy for Non-Small Cell Lung Cancer

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Study Product: *[^{18}F]-fluorothymidine (FLT)*

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List of Abbreviations

FLT 3'-deoxy-3'-[18F]-fluorothymidine (FLT)

PET positron emission tomography

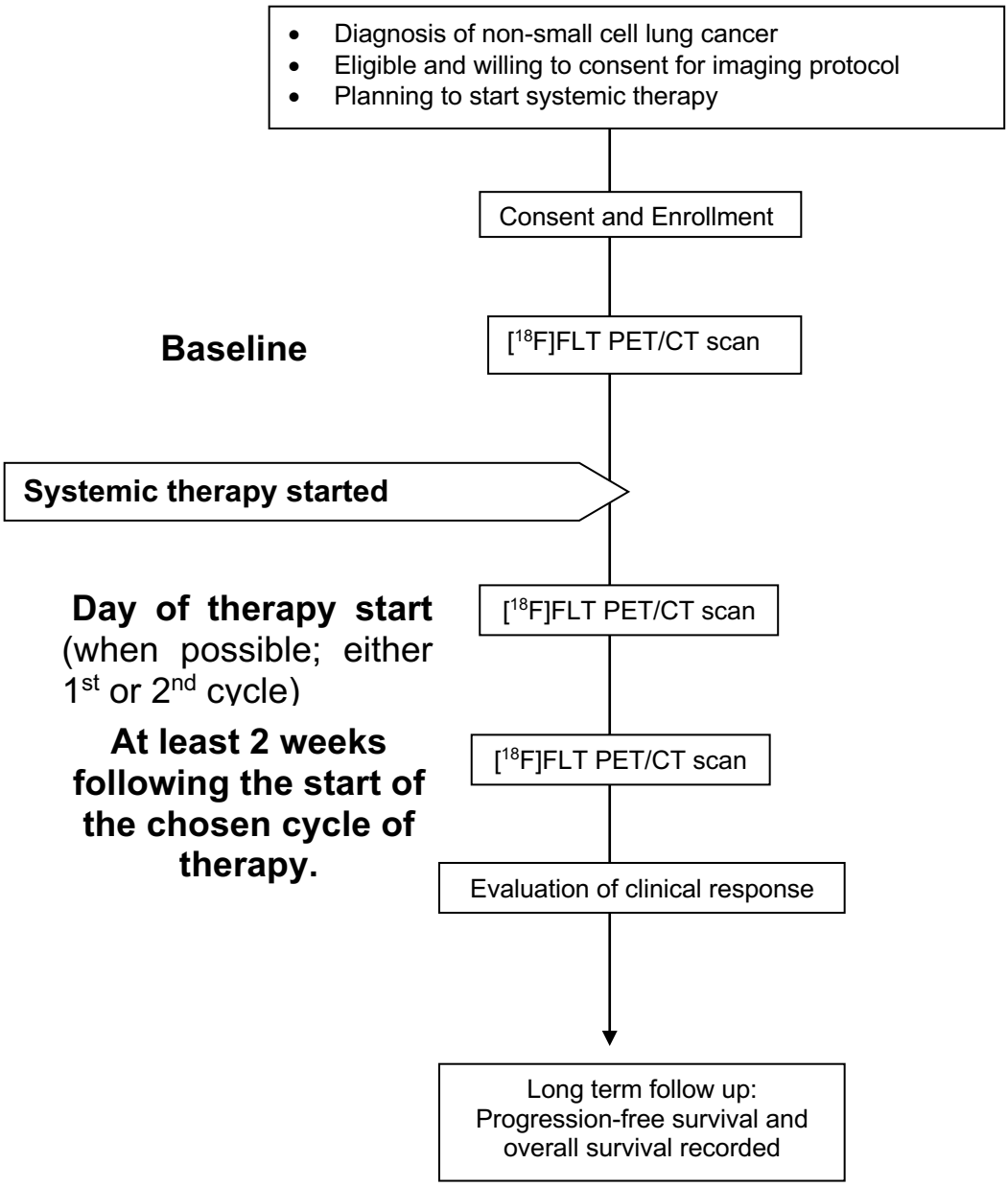
CT computed tomography

Study Summary

Title	Serial [18F]Fluorothymidine (FLT)PET/CT as a Biomarker of Therapeutic Response in Pemetrexed Therapy for Non-Small Cell Lung Cancer
Short Title	*Serial [18F]FLT PET/CT in NSCLC*
Protocol Number	
Study Design	Proof of concept study of serial [18F]fluorothymidine (FLT) as an imaging tracer for positron emisssion tomography (PET/CT) for imaging non-small cell lung cancer receiving pemetrexed therapy.
Study Duration	5-7 years
Study Center(s)	University of Pennsylvania
Objectives	Evaluation of [18F]fluorothymidine (FLT) as an imaging tracer for PET/CT imaging of non-small cell lung cancer(NSCLC). Compare pre- and post-treatment primary tumor uptake for FLT-PET/CT and correlate with clinical markers of response. PET/CT tumor metabolic response will also be correlated will to progression-free survival and overall survival. When tumor biopsy specimens are available, correlation will be made with the FLT-PET/CT metabolic response and histological markers including Ki67 staining, a measure of % nuclei actively engaged in cellular division.
Number of Subjects	Target accrual is 20 evaluable subjects; up to 30 subjects may be enrolled to reach this target goal
Diagnosis and Main Inclusion Criteria	Adult men and women of any ethnicity with NSCLC deemed unresectable by CT or PET imaging criteria and planning to start pemetrexed-based therapy.
Study Product	[^{18}F]-fluorothymidine ([^{18}F]FLT)
Statistical Methodology	Due to the exploratory nature of this pilot study of 30 patients, no tests of hypothesis are planned. Descriptive statistics for all variables of interest will be tabulated and graphically displayed by various classifications, in order to assess feasibility, justify sample size and aid in the planning of a more rigorous proposed subsequent hypothesis driven trial.

STUDY DESIGN

Serial [^{18}F]Fluorothymidine (FLT)PET/CT imaging as a measure of therapeutic response in patients with newly diagnosed small cell lung cancer



Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1 Background

1.1 Investigational Agent

3'-deoxy-3'-^{18}F fluorothymidine ([^{18}F]FLT) is a structural analog of the DNA nucleoside, thymidine (Figure 1). [^{18}F]FLT is a radiolabeled imaging agent that has been proposed for investigating cellular proliferation in normal bone marrow and changes with targeted radiotherapy with positron emission tomography (PET). Although [^{18}F]FLT is not incorporated into DNA, it is phosphorylated by *thymidine kinase*, an intracellular enzyme of the proliferation pathway that is upregulated in proportion to DNA synthesis. The phosphorylated, [^{18}F]FLT is trapped in the cell. Thus, FLT has potential as a marker of proliferating cells in proportion to the DNA synthesis rate. Therefore, [^{18}F]FLT is proposed as a radiolabeled PET imaging probe for *in vivo* assessment of cellular proliferation in malignant tumors. For complete information, please refer to the Investigator's Brochure for [^{18}F]FLT.

Figure 1.The chemical structures of FLT and thymidine

1.2 Rationale

BACKGROUND:

A need for early response assessment in NSCLC

Unresectable, locally advanced non-small cell lung cancer (NSCLC) has an extremely poor prognosis with a median survival time of 9 to 11 months[1]. Pemetrexed (PEM) along with a platinum-based antineoplastic (cisplatin or carboplatin), a 1st-line therapy for non-squamous NSCLC, has a response rate of 20-30%, meaning that that 70-80% of patients fail to respond to PEM-based therapy[2, 3]. Currently, assessment of response to therapy relies on CT changes in tumor size (CT Response Evaluation Criteria In Solid Tumors; RECIST 1.1)[4]; however, these measurements are conducted typically after 3 x 3-week cycles (9 weeks) of chemotherapy, often with associated delays, and thus patients with unresectable locally advanced

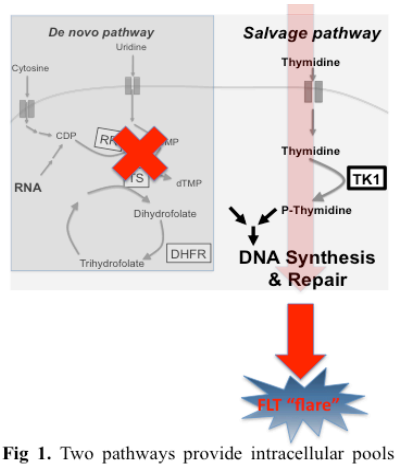


Fig 1. Two pathways provide intracellular pools of thymidine. Inhibition of the *de novo* synthetic pathway results in increased flux through the DNA salvage pathway, visible as a "flare" in FLT avidity. FLT is metabolized and trapped in the cell by the DNA salvage pathway

NSCLC now spend up to one-third of their expected life-span waiting just to see if their therapy is working. Moreover, the need for earlier response assessment is critical for clinical trials in NSCLC, where delays in response assessment lead to increased costs, unwarranted toxicities, and potential loss of lives.

[18F]FLT "flare" effect; exploiting a drug-drug interaction: DNA replication, whether in normal or cancer cells, requires thymidine[5], a fact exploited by a number of chemotherapeutic agents[6] including PEM[2, 3]. Two parallel pathways, the *de novo* and DNA salvage pathways **(Fig. 1),** source thymidine for the dividing cell. Pharmacologic inhibition of thymidylate synthase (TS) by PEM[2] inhibits *de novo* pathway activity, resulting in a transient (< 24 hours of start of therapy[7, 8]) burst of metabolism through the salvage pathway, an effect measurable with ^{18}F-thymidine ([^{18}F]FLT) as a tracer of the salvage pathway[7-13]; when salvage pathway activity increases, cells become increasingly more [^{18}F]FLT avid. Since TS over expression/over activity results in PEM resistance of NSCLC[14-17] we hypothesize that [^{18}F]FLT "flare," which represents a burst in tumor [^{18}F]FLT avidity induced by TS-inhibition, will predict successful PEM-based therapy in NSCLC and will permit determination of effective PEM-based therapy within 1 day of initiation. It is also apparent through the published clinical trials[12, 13, 18] on [^{18}F]FLT "flare", that choice of time point to measure [^{18}F]FLT "flare" may affect association with tumor metabolism. Since data suggests that imaging as soon as possible following starting TS inhibition, we will conduct [^{18}F]FLT PET/CT for measurement of "flare" as immediately as possible following completion of pemetrexed infusion (on same day of pemetrexed infusion), at the start of either the 1st or 2nd cycle of therapy.

Table 1. Studies of FLT for assessment of tumor response

	Primary Objective (Aims 1 and 2)	Secondary Objective (Aim 2)
Effect	FLT "Flare"	Decreased FLT avidity
Timing	<24 hours	2 weeks
Mechanism	Inhibition of thymidylate synthase by pemetrexed	Decreased proliferation of tumor cells
Specificity	Pemetrexed, 5-FU	Any chemotherapeutic

[18F]FLT as a measure of proliferation: [^{18}F]FLT is a reliable biomarker of proliferation[19] and using [^{18}F]FLT as a marker of tumor proliferative activity has been the most successful application of [^{18}F]FLT to date. As a chemotherapeutic begins to successfully impact the tumor metabolism, tumor proliferation generally falls below baseline. This post-therapeutic change in tumor avidity for [^{18}F]FLT has been demonstrated to predict therapeutic response within a few weeks from the start of therapy in a range of malignancies[20-24], including NSCLC[25]. However, [^{18}F]FLT PET/CT therapy response as a function of successful TS-inhibition, a commonly used therapeutic strategy, has not been well studied in NSCLC. By 2 weeks of therapy, any transient "flare" phenomenon will have dissipated and tumor avidity for [^{18}F]FLT should reflect overall tumor proliferation rate, which is expected to decrease with successful therapy. We hypothesize that a fall in NSCLC tumor avidity for [^{18}F]FLT after 2 weeks of PEM-based therapy will be predictive of therapy success and the stable to increased FLT avidity at 2 weeks will predict therapy failure. It is important to note that the [^{18}F]FLT "flare" response, a direct pharmacological effect, and the delayed decrease in [^{18}F]FLT avidity, a measured therapy effect on tumor metabolism, occur by different mechanisms (**Table 1**) providing complementary strategies for assessing

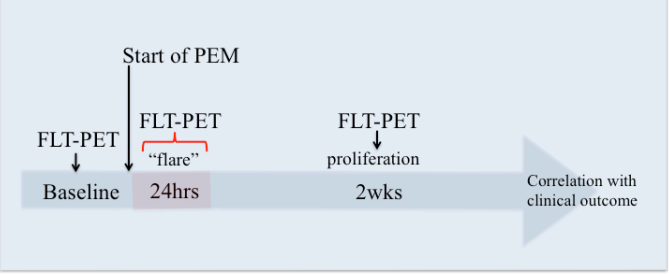


Fig 2: Correlative pilot study of FLT-PET as an imaging biomarker of TS inhibition following pemetrexed (PEM) therapy in NSCLC. Patients will obtain a baseline FLT scan. A second FLT scan will be performed within 24hrs of starting PEM in order to image the TS-inhibition mediated boost or "flare" in DNA salvage pathway activity. A third FLT scan will be performed at 2 weeks of therapy, after the transient FLT "flare" has dissipated, in order to measure PEM induced suppression of tumor proliferation.

tumor response. While the "flare" response is specific for TS inhibition (i.e. PEM), post-therapy decreased tumor avidity for [^{18}F]FLT should occur with any successful chemotherapy.

HYPOTHESES/OBJECTIVES: Our overarching hypothesis is that [^{18}F]FLT PET/CT will allow early assessment of therapeutic response in NSCLC. **Our primary objective** (**Table 1**) is to assess whether [^{18}F]FLT "flare", a transient drug-induced burst of DNA salvage pathway activity, is predictive of PEM-based therapy success. **Our secondary objective** is to determine whether [^{18}F]FLT PET/CT measured post-therapy changes in tumor proliferation correlate with PEM-based therapy clinical outcome.

Specific Aim 1: To conduct a proof-of-concept clinical study of tumor [^{18}F]FLT "flare" and [^{18}F]FLT -measured changes in tumor proliferation as predictors of NSCLC response to PEM-based therapy **(Fig 2).** *Hypothesis: NSCLC* [^{18}F]FLT *"flare" in the 1 day of a cycle of therapy and FLT proliferative response at least 2 wks later will predict clinical response to PEM-based therapy*.

1.3 Dose Rationale and Risk/Benefits Assessment

[^{18}F]fluorothymidine ([^{18}F]FLT) is an investigational imaging drug, which has been extensively used in oncologic PET imaging with no evidence of pharmacological effect. No adverse events have been reported in a large number of published studies of human experience for [^{18}F]FLT at the strength to be used for this study, doses ranging from 3-15 mCi have been reported in the literature with a large number of studies showing that a dose of approximately 5 mCi is safe and produces high quality images. The [^{18}F]FLT dose anticipated for this study will be 5.0 mCi ± 20%. Assuming a 70kg individual, the maximum concentration of [^{18}F]FLT would be expected to be equivalent to 0.29 ng-h/mL.

Risk/Benefit

The patients will undergo 3 study FLT-PET/CT examinations for the purposes of this study. [^{18}F]FLT is a positron emitting radiopharmaceutical. As such, it poses and intrinsic radiation exposure risk. However, when administered in low tracer doses as a PET imaging agent, as described in this protocol, this risk is felt to be extremely small. The organ and total body doses associated with FLT PET imaging are felt to be comparable to those associated with other widely used clinical nuclear medicine procedures.

To date approximately 2416 humans have been studied with [^{18}F]FLT in published literature. There have been no reported AEs associated with the used of [^{18}F]FLT and no adverse reactions are expected as a result of the IV injection of [^{18}F]FLT. We will continue to monitor this information ass the study progresses, including reviewing current literature and our in-house medical monitoring of this study.

There is potential with intravenous injections, including [^{18}F]FLT, for allergic reactions. This has not been observed in the published human exposure to date. The dose will be delivered intravenously by skilled clinical professionals and patients will be monitored for any signs or symptoms of allergic reaction by trained personnel for the duration of the PET procedure. The injection site may show bruising, bleeding, inflammation or infection.

No psychological, social or legal risk is expected. While loss of confidentiality is possible, it is felt to be very unlikely due to the small number of professionals involved in the study with knowledge of this information. All clinicians and research staff involved are well trained in HIPAA practices.

The risk to the patient is felt to be minimal with only the added radiation considered the likely risk posed to the patient and this radiation is considered acceptable. It is not unusual for oncologic patients to receive multiple radiological tests, including multiple PET/CT scans, in the course of clinical management. This study proposes the addition of 3 PET/CT scans to the patients care. There is potential benefit to the patient and to general society if FLT-PET/CT proves to be a useful imaging agent for the detection of response to cancer therapy. Pilot data from this study will be used to adequately power larger studies evaluating FLT-PET/CT as a measure of tumor response to therapy.

2 Study Objectives

Primary Objectives:

- Evaluation of [18F]fluorothymidine ([^{18}F]FLT) as an imaging tracer for PET/CT imaging of small cell lung cancer.
- Compare pre- and post-treatment primary tumor uptake for FLT-PET/CT and correlate with clinical markers of response.

Secondary Objectives:

- PET/CT tumor metabolic response will be correlated will to time-to-progression and survival.
- Correlate FLT-PET/CT uptake measures and histological markers, including Ki-67 staining, a measure of % nuclei actively engaged in cellular division when biopsy tissue is available.

3 Study Design

3.1 Study Design: This is a pilot study in patients with a diagnosis of non-small cell lung cancer with at least one lesion that is ≥ 1 cm on at least one type of standard imaging (e.g. CT, chest x-ray, MRI). Patients may participate in this study if they are greater than 18 years of age, most participants will be receiving care at the clinical practices of the University of Pennsylvania. Patients that meet the eligibility criteria will be approached about study participation regardless of race or ethnic background. We anticipate enrolling up to 30 participants with diagnosed non-small cell lung cancer beginning a new line of therapy who meet eligibility requirements for this study. The target accrual goal is to reach 20 evaluable patients who complete 2 or 3 FLT PET/CT scans, in order to reach this target we may enroll up to 30 subjects. Thus, accrual is estimated to occur over approximately 5 years.

Positron emission tomography (PET) imaging will be used to evaluate cell proliferation in sites of non-small cell lung cancer using the investigational radiotracer [^{18}F]FLT. Study imaging will be performed using a whole-body PET/CT scanner (Philips Medical Systems, Netherland). The protocol will be performed under the regulatory approval of the (Institutional Review Board) IRB and FDA IND.

A baseline FLT-PET/CT will be obtained no more than 1 week prior to initiation of the chosen cycle therapy to assess baseline, pre-therapy tumor proliferative activity. On the day of 1st infusion of pemetrexed therapy of either cycle 1 or 2 of therapy, a post-therapy FLT-PET/CT will be obtained, when possible. There may be patients that will be unable to obtain this 2nd FLT-PET/CT scan due to scheduling/feasibility issues and those patients will

forgo the 2nd FLT-PET/CT (designed to measure the FLT flare effect) and will go on to the 3rd scheduled FLT-PET/CT scan (designed to measure changes in tumor proliferation). If the 2nd FLT PET/CT scan is omitted the reason (e.g. scheduling limitations) will be documented in the CRF, this will not be considered a protocol deviation. The third FLT-PET/CT will be obtained at least 2 weeks following the start of the chosen cycle of therapy patients to assess for more distal effects of therapy on tumor proliferation.

For each PET scan static images from the skull base to upper thighs will be acquired approximately 60 minutes after infusion of [^{18}F] FLT. Imaging data will be processed as per standard protocol. The SUVmax and total uptake volume of the primary tumor will be calculated FLT-PET/CT scans. Comparison will also be made between the initial tumor uptake (SUV) and Ki-67 staining of the tumor nuclei which will be performed by the Pathology Core at the CHOP Research Institute when biopsy tissue is available.

The patient's active participation in this study will end following the completion of the last FLT-PET/CT scan. The patient's medical records will be accessed following for an estimated duration of 5-7 years following enrollment. This includes time for patient enrollment and clinical data collection. The patient's clinical information will be accessed from the HUP clinical charts, HUP clinical database, including Medview, and radiology databases in order to calculate time-to-progression and overall survival. For those whose are cared for by a non-HUP clinician during this period, this clinician will be contacted by phone for follow-up. The patients may also be contacted by phone for follow-up.

3.2 Study Design Exceptions: A study exception is a one time, intentional action or process that departs from the IRB and CTSRMC approved study protocol, intended for one occurrence. If the action disrupts the study progress, such that the study design or outcome (endpoints) may be compromised, or the action compromises the safety and welfare of study subjects, advance documented IRB and DSMC approval is required. For this in-house study with a Sponsor Monitor, approval will be obtained from the Study Sponsor prior to submitting the exception request to the IRB and DSMC.

3.3 Study Design Deviation: A study design deviation is a one time, unintentional action or process that departs from the IRB and DSMC approved study protocol, involving one incident and identified retrospectively, after the event occurred. If the impact on the protocol disrupts the study design, may affect the outcome (endpoints) or compromises the safety and welfare of the subjects, the deviation will be reported to the DSMC within 5 business days and the IRB within 10 business days.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Adult patients, at least 18 years of age
2. Histologically confirmed non-small cell lung cancer with at least one site of disease > 1 cm by at least one type of standard imaging (e.g. CT, chest x-ray, MRI)
3. Recommmended to start systemic therapy which includes pemetrexed and a platinum-based agent.
4. Participants must be informed of the investigational nature of this study and provide written informed consent in accordance with institutional and federal guidelines prior to study-specific procedures.

5. Participants must be willing and able to comply with scheduled visits and imaging procedures in the opinion of the investigator or treating physician.

4.2 Exclusion Criteria

1. Females who are pregnant or breast-feeding at the time of screening will not be eligible for this study. Female participants of child-bearing potential will have a urine pregnancy test at the time of the screening visit.
2. Patients with only a single site of primary lung cancer who have undergone or are recommended to undergo radiation therapy to that site will not be eligible, the inclusion of patients who may be undergoing radiation therapy to ancillary disease sites may be allowed to enter the study at the discretion of the PI if it is not felt to affect the ability to capture FLT information for at least one primary site of disease.
3. Patients who have undergone cancer surgery removing a significant portion of active disease, in the opinion of an investigator, within 2 months prior to study enrollment will be excluded.
4. Inability to tolerate imaging procedures in the opinion of the investigator or treating physician
5. Serious or unstable medical or psychological conditions that, in the opinion of the investigator would compromise the subject's safety or successful participation in the study.
6. Unwilling or unable to provide informed consent

4.3 Subject Recruitment and Screening

The patients will generally be offered enrollment at the time of their office visit to oncology at the Hospital of the University of Pennsylvania following determination of the line of therapy that is most appropriate. Study personnel or an investigator will present the patient with information regarding the study and enrollment. Enrollment into the study will be presented as optional and will not impact clinical management or choice of therapeutic intervention. It will be emphasized that enrollment in the study is not mandatory and that failure to enroll will not result in prejudice by the treating clinicians and will not affect patient care.

All patients being considered for the study and eligible for screening must sign an informed consent for the study prior to any study specific procedures. Following completion of the pretreatment assessments and confirmation of eligibility, patients may undergo the baseline [^{18}F]FLT PET/CT scan.

4.4 Subject Withdrawal

The criteria for enrollment must be followed explicitly. If a subject who does not meet enrollment criteria is inadvertently enrolled, that subject should be discontinued from the study. A subject may voluntarily discontinue participation in this study at any time. The investigator may also, at his or her discretion, discontinue the subject from participating in this study at any time. If a subject is prematurely discontinued from participation in the study for any reason, at any time, at either the investigator's discretion or the subject's request, an effort must be made to document the reason(s) why a subject fails to return to the study clinic for necessary visits or is discontinued from the study. The primary reason for discontinuing participation in the study may include, but is not limited to, one of the following:

- Withdrawal of consent for treatment or imaging protocol by patient
- Noncompliance with protocol, e.g., the patient fails to appear at one or more imaging procedures
- Development of an intercurrent illness, injury, or medical condition likely to interfere with subject safety, the overall assessment, or the required administration of study medication
- Pregnancy
- Development of any condition for which the investigator feels treatment withdrawal is justified
- Termination of the study

Follow-up information will be obtained for subjects who discontinue the study.

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety, behavioral, or administrative reasons. If a subject does not return for a scheduled visit, every effort should be made to contact the subject. In any circumstance, every effort should be made to document subject outcome, if possible.

If the subject withdraws from the study, and also withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data should be collected. The sponsor may retain and continue to use any data collected before such withdrawal of consent.

5 Investigational Agent

5.1 Description

3'-deoxy-3'-^{18}F fluorothymidine ([^{18}F]FLT) is a structural analog of the DNA nucleoside, thymidine. [^{18}F]FLT is a radiolabeled imaging agent that has been proposed for investigating cellular proliferation with positron emission tomography (PET). Although [^{18}F]FLT is not incorporated into DNA, it is phosphorylated by *thymidine kinase*, an intracellular enzyme of the proliferation pathway that is upregulated in proportion to DNA synthesis. The phosphorylated, [^{18}F]FLT is trapped in the cell. Thus, FLT has potential as a marker of proliferating cells in proportion to the DNA synthesis rate. Therefore, [^{18}F]FLT is proposed as a radiolabeled PET imaging probe for *in vivo* assessment of cellular proliferation in cancer. The drug is a clear solution that is stored at room temperature in a gray butyl septum sealed, sterile, pyrogen-free glass vial and has an expiration time of 8 hours. The injectable dose of [^{18}F]FLT for most studies will be ≤ 0.07 mCi/kg of fluorine-18. In the dose of [^{18}F]FLT, only a small fraction of the FLT molecules are radioactive. The amount of injected drug is ≤ 6.1 µg (≤ 25 nmol per dose) of FLT. There is no evidence that nonradioactive and radioactive FLT molecules display different biochemical behavior.

5.2 Preparation of Study Drug

The manufacturing of [^{18}F]FLT will occur in the Cyclotron Facility of the Department of Radiology at the University of Pennsylvania. This facility manufactures USP compliant radio-labeled compounds for human use on a daily basis. The drug manufacturing will be fully documented and controlled by a set of Standard Operating Procedures (SOPs) prepared by the University of Pennsylvania Cyclotron under IND #102,482. All standard hospital procedures will apply.

5.3 Study Drug Receipt and Administration: [^{18}F]FLT

[^{18}F]FLT will be delivered to the Nuclear Medicine Division of the University of Pennsylvania Medical Center by a trained Cyclotron team member in single dose vials according to the standard procedures outlined by the Cyclotron Facility. Once the drug has been delivered to the Nuclear Medicine Division all standard hospital procedures will apply for handling, processing, and destruction of any residual amounts if applicable. Prescribed dose will be drawn and accurately measured by the dose calibrator in the imaging facility and immediately administered by bolus injection to the patient under the direct supervision of a Nuclear Medicine Authorized User . The injectable dose of [^{18}F]FLT for most studies will be approximately 5 mCi ± 20%. In the dose of [^{18}F]FLT, only a small fraction of the FLT molecules are radioactive. [^{18}F]FLT is administered to subjects by intravenous injection of ≤ 10 mL. The injection will be followed by a saline flush. The infusion and imaging procedure will be terminated in any patient who exhibits anaphylaxis, significant dyspnea or chest pain.

6 Study Procedures

STUDY CALENDAR

	Pre-Study	Day of [^{18}F]FLT PET/CT (Baseline)[2]	Day of [^{18}F]FLT PET/CT (Scan 2)[3]	Day of [^{18}F]FLT PET/CT (Scan 3)[4]
Informed Consent	X			
Demographics	X			
Pregnancy Test[1]	X	X	X	X
[^{18}F]FLT Injection		X	X	X
Adverse Event Evaluation		X [5]	X [5]	X [5]

[1]A urine pregnancy test will be done at screening in all women of child-bearing potential. In addition, in patients that are unsure if they could be pregnant, a urine pregnancy test will be repeated before injection of FLT for females of childbearing potential on the day of the FLT-PET scan.
[2]Baseline FLT PET/CT should take place no more than 1 week prior to initiation of the chosen cycle of therapy
[3]Performed on the same day of initiation of either cycle 1 or 2 of systemic therapy, this scan is optional and may be omitted if it cannot be done on the day of therapy initiation
[4] Performed at least 2 weeks after initiation of the chosen cycle of therapy.
[5]Follow up may occur by telephone or in person, depending on the subject's schedule. Follow up should take place the next available business day after the FLT PET/CT scan (i.e. not a weekend or a holiday)

6.1 Initial pre-study screening prior to [^{18}F]FLT PET/CT scan

The investigator will provide for the protection of the subjects by following all applicable regulations. These regulations are available upon request from the sponsor. The sponsor and IRB must review the informed consent form used during the informed consent process, and it must be available for inspection.

Before any procedures specified in this protocol are performed, a subject must:

- Be informed of all pertinent aspects of the study and all elements of informed consent.
- Be given time to ask questions and time to consider the decision to participate.
- Voluntarily agree to participate in the study.
- Sign and date an IRB/IEC-approved informed consent form..

The following additional patient data will be obtained: demographics, including gender, date of birth and race, histologic diagnosis, age at diagnosis, height and weight. Correlative pertinent imaging including CT, MRI, FDG PET/CT or bone scan may be reviewed. For patients referred from outside the University of Pennsylvania health system, patient records and biopsy material and imaging as necessary, will be reviewed to determine eligibility for the study. For women of childbearing potential a urine pregnancy test will be performed at the screening visit.

6.2 [^{18}F]FLT PET/CT Imaging Visit

The following procedures will be done at each imaging session

- Weight will be recorded
- Injection of [^{18}F]FLT
- Prior each FLT-PET/CT scan, female patients of child-bearing potential may have a urine pregnancy test if the patient is unsure about whether she might be pregnant.

All women of child-bearing potential will be asked on the day of the PET/CT scan if they might be pregnant this is a standard question for all patients who will be undergoing PET/CT scans due to the radiation exposure associated with the scan. If the patient is unsure about whether she might be pregnant then a urine pregnancy test will be performed prior to the injection of [^{18}F]FLT. The patient will be made comfortable in a preparatory room. Approximately 5 mCi ± 20% of [^{18}F]FLT will be administered by bolus injection to the patient under the direct supervision of a Nuclear Medicine Authorized User. A lesser activity may be injected if, in the opinion of a nuclear medicine authorized user, complete imaging data could be generated. Prior to positioning on the PET/CT scanner the patient will be allowed to urinate if necessary.

All patients will undergo a skull base to mid-thigh PET/CT scan starting at approximately 60 minutes after [^{18}F]FLT injection. A brief low-dose CT scan will be acquired according to standard PET/CT imaging procedures, this is used for attenuation correction and anatomical localization of findings in the PET scan. This can be performed either before or after the PET transmission scan. There are no separate diagnostic CT scans performed as part of this research.

A second [^{18}F]FLT PET/CT scan will be performed, when possible, on the same day of the initiation of either the 1st or 2nd cycle of therapy for lung cancer. This scan may not take place in all patients.

A third [^{18}F]FLT PET/CT scan will be performed at least 2 weeks after the initiation of the chosen cycle of therapy.

Adverse events that are grade 3 or higher will be recorded for the period up to 10 hours post injection of the radiotracer. Research personnel will conduct follow up by telephone or in person, depending on the subject's schedule. Follow up should take place the next available business day (i.e. not a weekend or a holiday).

6.3 [^{18}F]FLT PET/CT Image Analysis

Static images will be reconstructed using standard procedure and analyzed by visual inspection and standardized uptake values (SUV) analysis. SUVmax will be recorded for an index lesion, usually a primary lung lesion, follow up scans will use the same index lesion to compare change in SUVmax from baseline.

6.4 *Post-FLT-PET/CT Follow-up*

The patient will be asked to allow long term follow up for the duration of their cancer care. Follow up will primarily occur through review of HUP clinical charts, HUP clinical database, including Medview, and radiology databases in order to estimate time-to-progression or death. For those whose are cared for by a non-HUP clinician during this period, this clinician will be contacted by phone for follow-up. The patients may also be contacted by phone for follow-up**.**

7 Statistical Plan

7.1 Sample Size Determination

With n=20 subjects, a 95% exact confidence interval for the response rate will be as wide as 0.456 if the response rate is 50% (10/20), or as narrow as 0.305 if the response rate is 10% (2/20). We will test whether the mean SUVmax is the same in subjects who respond and those who do not. With 10 responses, a two-sample t test comparing the means with type I error rate 5% will have power at least 80% if the difference in means is at least 1.32 standard deviations (SDs). If there are 5 responders and 15 non-responders, the power will be at least 80% if the difference in means is at least 1.53 SDs.

7.2 Statistical Methods

This is a pilot study of the FLT radiotracer to image tumor proliferative response of cancer to therapeutic intervention. We will characterize and examine associations among the following variables of interest: max standard uptake value (SUVmax); total tumor uptake volume; Ki-67 staining; progression-free survival (PFS, measured as time from treatment to the earlier of progression or death); and overall survival (OS, time from treatment to death). Time-to-event variables will be measured from the time of initiation of treatment, and will be censored at the data of last follow-up.

Due to the exploratory nature of this pilot study, we do not anticipate having power to detect significant associations unless effects are very large. For continuous uncensored measures,

estimates will include means, standard deviations, 95% confidence intervals, medians and inter-quartile range, which will tabulated and graphed by classification variables of interest. We will estimate distributions of PFS and OS by the Kaplan-Meier curve. We will tabulate descriptive statistics for all variables of interest, using the data to aid in the planning of a more rigorous subsequent hypothesis-driven trial. Associations among relevant variables will be explored through the use of Spearman's correlation coefficient, bivariate plots, and the logrank test, as appropriate. We will conduct statistical analyses using STATA software.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- Is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 10 hours following the administration of the study radiotracer [^{18}F]FLT.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each FLT PET/CT imaging appointment, the investigator must seek information on adverse events specific to imaging procedures or injection of FLT by specific questioning and, as appropriate, by examination. Information on all adverse events that are grade 3 or higher should be recorded immediately in a source document or the medical record. All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the adverse event reporting period, 10 hours following injection of [^{18}F]FLT must be recorded if they are grade 3 or higher. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the adverse event reporting period must be followed up to determine the final outcome. Any serious adverse event that occurs after the adverse event reporting period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others (see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study drug ([^{18}F]FLT)

8.3.1 Investigator reporting: notifying the study sponsor

Any study-related unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event, must be reported initially to the study sponsor (The Department of Radiology IND office) by email within 24 hours of when the investigator becomes aware of the event. To report such events, a description and summary of the event must be completed by an investigator or designee, signed by the investigator and faxed or emailed to the study sponsor within 48 hours of when the investigator becomes aware of the event. The investigator will keep a copy of this SAE summary report on file at the study site. Further information about follow up or resolution of serious adverse events should be provided promptly to the study sponsor as it is available and/or upon the request of the sponsor.

8.3.2 Investigator reporting: notifying the Penn IRB

This section describes the requirements for safety reporting by investigators who are Penn faculty, affiliated with a Penn research site, or otherwise responsible for safety reporting to the Penn IRB. The University of Pennsylvania IRB (Penn IRB) requires expedited reporting of those events related to study participation that are

unforeseen and indicate that participants or others are at increased risk of harm. The Penn IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The Penn IRB requires researchers to submit reports of the following problems within 10 working days from the time the investigator becomes aware of the event:

- Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

  Unexpected (An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)

  AND

  Related to the research procedures (An event is "related to the research procedures" if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.)

  Reporting Process
  Unanticipated problems posing risks to subjects or others as noted above will be reported to the Penn IRB using the form: "Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

  Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

8.3.3 Investigator reporting: notifying the Cancer Center DSMC

AEs will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 for reporting to the DSMC:

- All grade 3 or higher events within five days of knowledge
- All unexpected deaths within 24 hours of knowledge
- All others deaths within 30 days of knowledge

8.3.4 Sponsor reporting: Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days***
  Any study event that is:
  – associated with the use of the study drug
  – unexpected,
  – fatal or life-threatening, and

- ***Within 15 calendar days***
  Any study event that is:
  – associated with the use of the study drug,
  – unexpected, and
  – serious, but not fatal or life-threatening
  -or-
  – a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  – suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements
Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

8.3.5 Sponsor reporting: Notifying participating investigators

It is the responsibility of the study sponsor to notify all participating investigators, in a written IND safety report, of any adverse event associated with the use of the drug that is both serious and unexpected, as well as any finding from tests in laboratory animals that suggest a significant risk for human subjects. Additionally, sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

8.4 Medical Monitoring

It is the responsibility of the Principal Investigator, Dr. Katz and the nuclear medicine AUs overseeing injection of the investigational agent, to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 11 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

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9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why

- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive).

All research personnel associated with this study have completed the University of Pennsylvania's Patient Oriented Research Training Program as well as HIPAA Compliance Training. Trained staff will assess eligibility, introduce the study rationale, procedures, study risks, and collect the combined informed consent/HIPAA authorization form. The study team will work to uphold the privacy of the participants in several ways. Communications made among study staff regarding participants will use ID numbers whenever possible and minimize the use of patient name or other identifying information except when necessary for conduct of the study. Paper-based records will be kept in a secure location and only be accessible to personnel involved in the study. Computer-based files will only be made available to personnel involved in the study through the use of access privileges and passwords. Whenever feasible, identifiers will be removed from study-related information. In data analysis sets, we will use ID numbers and/or patient initials only.

Precautions will be applied to protecting subject privacy and the protected health information detailed below:

1. Name
2. Address
3. Date of Birth
4. Telephone Number
5. Email address
6. Emergency contact number, name, and relationship
7. Medical Record Number
8. Health Plan ID numbers

Data will be accessible to the study investigators, all study staff, Department of Radiology IND office representatives, Radiation Research Safety Committee members, UPenn IRB and Office of Human Research, and the FDA (if desired).

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black or blue ink. If any entry error has been made, to correct such an error, a single straight line will be drawn through the incorrect entry and correct data entered above it. All such changes will be initialed and dated. For clarification of illegible or uncertain entries, the clarification will be printed above the item, then the item will be initialed and dated.

9.4 Records Retention

The investigator will retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in the USA or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The Principal Investigator is responsible for assigning staff for preliminary monitoring of study files. Staff members are required to review all subject binders for completion and accuracy. This review will be documented. The investigative team will meet after Subject 1 to review the study flow and to discuss any issues that occurred. The study team will also determine at this time if any modifications to the protocol are necessary. This meeting will be documented. The Sponsor will also supply a Departmental monitor (assigned through the Departmental IND office), and DOD medical monitor, Daniel Pryma, MD, for the below plan.

The role and responsibilities of the Departmental Research Monitor listed in the protocol include:

a) May discuss the protocol with the investigators and consult with others outside the study about the research.

b) Shall report to the Sponsor who will then be obligated to ensure the Principal Investigator properly reports to the IRB, the Abramson Cancer Center CTSRMC (Clinical Trials Scientific Review and Monitoring Committee). Study monitoring ensures that necessary steps are taken to protect the safety and well-being of subjects.

c) The study Departmental Research monitor (on behalf of the Sponsor) will ensure that the Principal Investigator communicates with the UPENN IRB and CTSRMC in accordance with the reporting structure of those entities in the case of a Serious Adverse Event (SAE). The study Departmental Research monitor will ensure the final determination is properly documented and reported to all necessary regulatory bodies as well as the USAMRMC ORP HROP.

d) The study Departmental Research monitor will assist the Principal Investigator if needed to ensure all SAE's are properly documented. This will including reviewing all

reports if request, on behalf of the Sponsor prior to submitting to the regulatory committees (IRB, CRSRMC).
e) The study Departmental Research monitor or will assist with communication between the Sponsor and the Principal Investigator as needed.

The role and responsibilities of the study DOD Medical Research Monitor listed in the protocol include:

a) May discuss the protocol with the investigators, interview subjects, and consult with others outside the study about the research.

b) Shall have the authority to stop the protocol, remove subjects from the protocol, and take any necessary steps to protect the safety and well-being of subjects until the IRB can assess the Monitor's report.

c) Shall have the responsibility to promptly report their observations and findings to the IRB or other designated official if in disagreement with the assessment of the PI.

d) Is required to review all unanticipated problems involving risks to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the research monitor must comment on the outcomes of the event or problem and in the case of a serious adverse event or death, comment on the relationship to participation in the study.

e) The research monitor must also indicate whether he/she concurs with the details of the report provided by the principal investigator. Reports for events determined by either the investigator or research monitor to be possibly or definitely related to participation and report of events resulting in death must be promptly forwarded to the USAMRMC ORP HRPO.

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all requested study-related documents and facilities if necessary.

Frequency

Enrollment will be complete when up to **20** evaluable subjects are enrolled into the trial. Monitoring sessions will be conducted periodically throughout the study as described below.

- The **first monitoring session** will occur after the first two subjects have been enrolled.
- A **second monitoring session** will be conducted when approximately 50% of the subjects have been enrolled.
- A **third monitoring session** will be conducted after 100% of the subjects have been enrolled. This visit may be conducted after the subjects have completed the study and can also serve as the close-out monitoring visit.

Data Review

Case Report Forms (CRFs) for the first 2 subjects will be 100% source data verified. In addition, the CRFs for at least an additional 3 randomly selected subjects will be 100% source data verified.

If a high error rate is noted during the data review, this monitoring plan may be revised and more subject files may be 100% source data verified

Additional subjects/events to be reviewed:

All subjects who discontinued due to serious adverse events will be reviewed for key safety and efficacy data.

10.2 Regulatory Documents Reviewed

The Regulatory Documents will be maintained in the Regulatory Binder and electronically. The Regulatory Binder may be reviewed by the monitor during any visit. The monitor will review the regulatory binder for completeness.

10.3 Documentation of the Monitoring Visit

Monitoring Report

All monitoring visits will be documented on the Monitor's Report and Visit Checklist. The original report for each session will be filed in the Sponsor section of the Regulatory Binder. The Sponsor will also retain a copy of this report.

10.4 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

12 Study Finances

12.1 Funding Source

This study will be funded through the Department of Radiology and through funds through currently funded NIH and DOD grants and pending grant applications.

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of Pennsylvania investigators will follow the applicable University conflict of interest policies.

12.3 Subject Stipends or Payments

We will pay for the cost of parking, if not already covered through the hospital, on the day of each of the 2-3 PET/CT scans. In addition, we will reimburse the patient a maximum of $1000 ($50- completion of 1st scan, $50-completion of 2nd scan, $900- completion of 3rd scan) after the completion of the last FLT-PET/scan to cover the inconvenience of time and effort spent in participation of this study.

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