Study Title: 18-FLT PET/MR Imaging to Predict Graft Failure and Graft Versus Host Disease in Bone Marrow Transplant Patients

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LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

LCCC1714 : 18-FLT PET/MR Imaging to Predict Graft Failure and Graft Versus Host Disease in Bone Marrow Transplant Patients

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LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
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LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

PROTOCOL AMENDMENT #4

LCCC1714 : 18-FLT PET/MR Imaging to Predict Graft Failure and Graft Versus Host Disease in Bone Marrow Transplant Patients

AMENDMENT INCORPORATES:

____ **Editorial, administrative changes**
__X__ **Scientific changes (IRB approval)**
_____ **Therapy changes (IRB approval)**
____ **Eligibility Changes (IRB approval)**

Summary of Changes –February 7th, 2019

1. Changing the post transplant window to allow the first scan to occur within +/- 5 days instead of +/- 3 days from their day 25 post transplant.

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PROTOCOL AMENDMENT #3

LCCC1714 : 18-FLT PET/MR Imaging to Predict Graft Failure and Graft Versus Host Disease in Bone Marrow Transplant Patients

AMENDMENT INCORPORATES:
____ Editorial, administrative changes
____ Scientific changes (IRB approval)
____ Therapy changes (IRB approval)
__X__ Eligibility Changes (IRB approval)

Summary of Changes – September 4th, 2018

1. Removing the BMI exclusion criteria.

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PROTOCOL AMENDMENT #2

LCCC1714 : 18-FLT PET/MR Imaging to Predict Graft Failure and Graft Versus Host Disease in Bone Marrow Transplant Patients

AMENDMENT INCORPORATES:

_X_ Editorial, administrative changes
____ Scientific changes (IRB approval)
____ Therapy changes (IRB approval)
____ Eligibility Changes (IRB approval)

Summary of Changes – June 18th, 2018

1. Grace David added a new coordinator
2. Removed Rebecca Rainer, who no longer works for BRIC

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UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

PROTOCOL AMENDMENT #1

LCCC1714 : 18-FLT PET/MR Imaging to Predict Graft Failure and Graft Versus Host Disease in Bone Marrow Transplant Patients

AMENDMENT INCORPORATES:

_X_ Editorial, administrative changes
____ Scientific changes (IRB approval)
____ Therapy changes (IRB approval)
____ Eligibility Changes (IRB approval)

Summary of Changes – February 8th, 2018

1. Added Rebecca Rainer as research assistant to the study
2. Removed Soma Prum, who no longer works for BRIC

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LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

LCCC XXXX: 18-FLT PET/MR Imaging to Predict Graft Failure and Graft Versus Host Disease in Bone Marrow Transplant Patients

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Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator (PI) Name:_Yueh Lee, MD, PhD_____

PI Signature: _____

Date:_____

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1.0 BACKGROUND AND RATIONALE

1.1 Study Synopsis

This is a non-randomized, prospective pilot study exploring the use of fluorothymidine (FLT)-PET-MRI in the evaluation of allogeneic bone marrow transplant patients to potentially predict engraftment success, malignancy relapse, and the development of graft versus host. A total of 12 patients who have undergone allogeneic bone marrow transplantation will undergo FLT-PET-MRI imaging on two separate occasions. The first scan will occur immediately after initial neutrophil count recovery on day +25. The second scan will occur on transplant day +60 by which time stable count recovery should have occurred. The findings on the first scan will be correlated with clinical outcomes and pathological results on transplant day +35, and the findings on the second scan will be correlated with clinical outcomes and pathological results on day +100. The day +35 and +100 time points were chosen because allogeneic transplant patients at our institution undergo a restaging bone marrow biopsy and aspirate with donor/recipient chimerism studies around these days as part of our usual standard of care. In addition to the 12 allogeneic transplant patients, 3 patients undergoing autologous stem cell transplant will also be imaged at the same two time points in order to determine how much of the FLT signal observed after allogeneic transplant is unique to that population and the result of allo-antigen driven T cell expansion.

1.1.1 Allogeneic HSCT and Graft-versus-Host Disease

Allogeneic HSCT is potentially curative for numerous high risk hematologic malignancies and offers several advantages over traditional chemotherapy. First, higher doses of cytotoxic chemotherapy and/or irradiation can be given since patients are subsequently rescued from the severe myelosuppression induced by the pre-transplant conditioning regimen by the infusion of healthy hematopoietic stem cells. Second and perhaps more importantly, mature T cells contained in the graft are able to mount immune responses against residual cancer cells surviving the conditioning regimen due to major and/or minor MHC disparities between the donor and recipient. Unfortunately, the allo-immune responses driving the GVL effect are typically not specific for malignant cells. As a consequence, donor immune cells attack normal host tissues resulting in a process known as acute graft-versus-host disease (GVHD). Acute GVHD is primarily T cell driven, usually occurs within the first few months after transplant, and results in skin rash, diarrhea, cholestatic liver damage, and, on occasion, acute lung injury.

The process by which donor T cells initiate the GVHD process has been extensively studied in animal models, and was reviewed previously.[1] In brief, naïve, "conventional" donor T cells (T_{cons}) traffic into recipient secondary lymphoid tissue

(spleen, lymph nodes, Peyer's patches) early after transplant, and undergo activation and expansion upon exposure to allo-antigens presented by *host* antigen presenting cells.[2] Donor T cells then migrate from host secondary lymphoid tissue (SLT) to peripheral GVHD "target organs", where they elicit tissue damage. The efficient homing of conventional donor T cells into recipient SLT appears to be *absolutely required* for GVHD pathogenesis in mice as recipient animals lacking all lymphoid tissue fail to develop GVHD following allogeneic transplantation.[3,4] The relationship between donor immune cell expansion within host lymphoid sites and GVHD incidence in humans, however, has not been described.

Allogeneic HSCT patients are at risk for not only GVHD after transplant, but also poor stem cell engraftment and/or graft rejection which can ultimately lead to disease relapse. Currently there are no reliable methods for predicting in advance which allogeneic transplant patients are destined to develop graft failure/recurrent disease or GVHD. If, however, a radiographic approach could be developed to predict one or both of these outcomes, this would be extremely useful clinically. Both processes are modulated by immunosuppressive medications. Specifically, GVHD is prevented and treated with increased immunosuppression and graft failure is treated in the opposite manner. If an imaging modality could prospectively indicate the development of either of these processes during their earliest stages, this would allow the transplant physician to proactively tailor a patient's immunosuppression in order to hopefully improve transplant outcomes. Similarly, if an isolated site of disease relapse could be identified early, this could lead to directed tissue biopsy and the early initiation of pre-emptive chemotherapy and/or radiotherapy.

The current proposal explores the use of a novel imaging modality, FLT PET/MRI, to correlate allogeneic transplant outcomes with FLT and MRI findings during early stem cell engraftment and at a later time point following stable count recovery. Specifically, we will determine if the strength of the early FLT signal within the bone marrow correlates with engraftment success and if isolated areas of cellular proliferation within the marrow at a later time point might predict for leukemia relapse. In addition, based on the important role that host lymphoid tissues are known to play in GVHD pathogenesis in mice, we will determine if the FLT signal within host SLT after transplant can predict for the development of GVHD in human BMT patients. Because FLT imaging by itself cannot distinguish between bone marrow engraftment/proliferation and the allo-immune driven T cell expansion that ultimately results in GVHD, we will image <u>autologous</u> transplant patients as a comparator arm. Autologous HSCT like allogeneic transplantation involves the administration of very high doses of chemotherapy to high risk cancer patients in order to achieve better tumor kill. However, in this situation patients are administered their own cryopreserved stem cells to reconstitute the ablated hematopoietic system. Under those circumstances there is no allo-immune reactivity to drive T cell activation and expansion after transplant, and as a result there is no GVHD in the autologous transplant setting. Thus, these patients will help us to

elucidate how much of the FLT signal seen in the allogeneic setting is the result of allo-immune driven T cell expansion.

FLT-Imaging: Fluorodeoxyglucose (FDG)-PET detects the accumulation of FDG in glucose-avid tissue, and is a measure of overall metabolic activity. FDG is relatively simple to synthesize, has a long half-life (approximately 2 hours), and has a well-understood mechanism of uptake, making it the most commonly used radiotracer in oncology.[5,6]

The diagnostic value of FDG-PET, however, is limited by its lack of specificity. There has been great interest in taking advantage of the various unique properties of tumor cells in PET imaging. Tumor and expanding inflammatory cells show increased DNA synthesis during cellular proliferation, making the DNA synthetic pathway an attractive target for their visualization.

FLT, an analogue of the pyrimidine thymidine, has recently become a PET imaging agent of interest due to its potential to image cellular proliferation. Briefly, FLT is taken up by specific nucleoside transporters on the cell membrane via facilitated diffusion, and subsequently phosphorylated by tyrosine kinase 1 (TK1). Once phosphorylated, FLT is metabolically trapped within the cell. Thus, 18F labeled FLT detection via PET provides a measure of TK1 activity, which is closely linked to the salvage pathway of DNA synthesis and cellular proliferation.[7] 18F-FLT and 18F-FDG PET have the shared advantage of utilizing an 18F radiotracer with a 110 minute half-life, making 18F-FLT PET feasible in diagnostic PET sites without dedicated on-site cyclotrons. However, 18F-FLT PET addresses the limitations seen with 18F-FDG PET. Specifically, decreased background "noise" is seen with 18F-FLT PET, and confounding factors such as muscle uptake do not occur. The invention of PET/MR brings together the functional imaging capabilities of PET with the exquisite soft tissue discrimination of MR.

1.1.2 FLT-Imaging in hematologic malignancies

Previous studies have explored the use of FLT-PET imaging to evaluate bone marrow engraftment in both preclinical animal models and human autograft patients, and demonstrate the overall feasibility of the imaging approach in the HSCT setting. In work by Awasthi et al. a FLT signal was detectable in the bone marrow compartment of Wistar rats within 4 days of syngeneic HSCT, and was much more sensitive for early marrow engraftment than traditional FDG-PET imaging.[8] In work by Woolthuis et al. the authors imaged 16 myeloma or lymphoma patients undergoing autologous HSCT transplant following high dose chemotherapy and compared them to non-transplanted controls .[9]There, the investigators observed an enhanced FLT signal in the marrow compartment of transplant patients, with a relatively uniform distribution primarily seen in the spine and to a lesser extent the pelvis and proximal long bones. Conversely, in work by Agool et al. patients with various active hematologic malignancies underwent FLT-PET imaging prior to therapy.[10] There, the FLT distribution

appeared to be more heterogeneous in appearance, with patchy areas of increased tracer uptake noted in patients with multiple myeloma, aplastic anemia, and myelodysplastic syndrome. Collectively, these data suggest that FLT-PET imaging is a sensitive tool for evaluating overall marrow recovery after HSCT, and that residual FLT activity as well as its overall distribution pattern after transplant could be a useful tool for monitoring for early disease relapse. Importantly, however, none of these publications evaluated allogeneic transplant patients and none correlated FLT-PET imaging results with the subsequent development of GVHD, graft failure, or disease relapse. Furthermore, none have attempted to correlate FLT-PET results with simultaneous MRI findings which could also be used to predict engraftment success after transplant.

2.0 STUDY OBJECTIVES AND ENDPOINTS

Primary Objectives

2.1.1 To compare the overall FLT-PET bone marrow signal on transplant day +25 between allogeneic stem cell transplant recipients who do and do not go on to achieve complete donor bone marrow reconstitution by transplant day +35.

2.1.2 To compare the overall FLT-PET signal intensity within host secondary lymphoid sites on transplant day +60 between allogeneic stem cell transplant recipients who do and do not develop acute GVHD by transplant day +100.

2.2 Secondary Objectives

2.2.1 To compare the overall FLT-PET bone marrow signal on transplant day +60 between allogeneic stem cell transplant recipients who do and do not achieve complete donor bone marrow reconstitution by transplant day +100.

2.2.2 To compare the overall FLT-PET signal intensity within host secondary lymphoid sites on transplant day +25 between allogeneic stem cell transplant recipients who do and do not develop acute GVHD by transplant day +100

2.2.3 To evaluate differences in FLT uptake within the bone marrow and secondary lymphoid tissues in patients undergoing autologous HSCT versus allogeneic HSCT

2.2.4 To correlate the strength of the FLT-PET signal within the bone marrow on transplant day +25 with the rate of transfusion independence on transplant day +35 in allogeneic stem cell transplant recipients

2.2.5 To correlate the strength of the FLT-PET signal within the bone marrow on transplant day +25 with bone marrow cellularity on transplant day +35 in allogeneic stem cell transplant recipients.

2.2.6 To correlate the strength of the FLT-PET signal within the bone marrow on transplant day +60 with the rate of transfusion independence on transplant day +100 in allogeneic stem cell transplant recipients

2.2.7 To correlate the strength of the FLT-PET signal within the bone marrow on transplant day +60 with bone marrow cellularity on transplant day +100 in allogeneic stem cell transplant recipients

2.2.8 To evaluate if isolated or asymmetric foci of increased FLT within the bone marrow or lymph nodes on transplant day +60 are associated with the incidence of disease relapse by day +100 in allogeneic stem cell transplant recipients

2.2.9 To correlate the strength of the FLT-PET signal within the bone marrow on transplant day +25 and on day +60 with MRI findings suggestive of engraftment in allogeneic stem cell transplant recipients

2.2.10 To evaluate the association of the overall FLT-PET signal intensity within host secondary lymphoid sites on transplant day +60 with the overall incidence of acute graft versus host disease and malignancy relapse over the first transplant year

2.3 Primary Endpoints

2.3.1 The overall FLT-PET bone marrow signal will be defined as the average maximum FLT SUV within the vertebrae, pelvis, and bilateral femurs measured at the time of early engraftment. This scan will be obtained on transplant day +25 +/- 5 days. Complete donor reconstitution will be defined as chimerisms ≥ 95% within the CD3 and unfractionated compartments on bone marrow biopsy/aspirate obtained on transplant day +35

2.3.2 The overall FLT-PET signal within host secondary lymphoid tissue will be defined as the average maximum FLT SUV within the spleen, cervical lymph nodes, axillary lymph nodes, inguinal lymph nodes, and mesenteric lymph nodes. This scan will be obtained on transplant day +60 +/- 5 days. Acute GVHD will be diagnosed either clinically or preferably pathologically within any target organ by transplant days +100.

2.4 Secondary Endpoints

2.4.1 The overall FLT-PET bone marrow signal will be defined as the average maximum FLT SUV within the vertebrae, pelvis, and bilateral femurs measured on transplant day +60. Complete donor reconstitution will be defined as chimerisms ≥ 95% within the CD3 and unfractionated compartments on bone marrow biopsy/aspirate obtained on transplant day +100

2.4.2 The overall FLT-PET signal within host secondary lymphoid tissue will be defined as the average maximum FLT SUV within the spleen, cervical lymph nodes, axillary lymph nodes, inguinal lymph nodes, and mesenteric lymph nodes

on transplant day +25. Acute GVHD incidence will diagnosed either clinically or preferably pathologically within any target organ by transplant day +100.

2.4.3 The overall FLT-PET signal within recipient secondary lymphoid tissue (average maximum FLT SUV within the spleen, cervical lymph nodes, axillary lymph nodes, inguinal lymph nodes, and mesenteric lymph nodes) and the bone marrow (average maximum FLT SUV within the vertebrae, pelvis, bilateral femurs) in autologous transplant recipients will be compared to the corresponding overall FLT-PET signals from allogeneic transplant recipients on transplant days +25 and +60.

2.4.4 Transfusion independence will be defined as not having received a packed red blood cell (PRBC) or platelet transfusion within the 7 days preceding the day +35 or the day +100 time point

2.4.5 Bone marrow cellularity will be defined in a dichotomous manner as either hypocellular or normocellular/hypercellular based on the bone marrow pathology report from the day +35 and day +100 bone marrow biopsies.

2.4.6 The average FLT SUV in the vertebral column will be compared to the quantitative T1 and Diffusion Weighted Imaging (DWI) signals in the vertebral column by MRI. The average FLT SUV in the pelvis will be compared to the quantitative T1 and Diffusion Weighted Imaging (DWI) signals in the pelvis by MRI. The average FLT SUV in the bilateral femurs will be compared to the quantitative T1 and Diffusion Weighted Imaging (DWI) signals in the bilateral femurs by MRI.

3.0 PATIENT ELIGIBILITY

3.1 Inclusion Criteria

3.1.1 Patients undergoing allogeneic bone marrow transplant for acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), or myelodysplastic syndrome

3.1.2 Allogeneic transplant patients receiving either a fully myeloablative or reduced intensity chemotherapy +/- total body irradiation (TBI) conditioning regimen are eligible.

3.1.3 Allogeneic transplant patients receiving stem cells from a matched related, matched unrelated, mismatched unrelated, mismatched related (including haplotype matched) donors are eligible

3.1.4 Allogeneic transplant patients must be in a complete morphologic remission prior to transplant

3.1.5 Patients undergoing autologous bone marrow transplant for multiple myeloma

3.1.6 Myeloma patients must have achieved at least a very good partial remission prior to transplant and exhibit fewer than 10% plasma cells in their pre-transplant marrow biopsy

3.1.7 $\geq$ 18 years of age

3.1.8 Able to provide informed consent

3.1.9 Negative urine pregnancy test in women of child-bearing potential

3.2 Exclusion Criteria

Subjects meeting any of the exclusion criteria at baseline will be excluded from participating in this study.

3.2.1 Any woman who is pregnant or has reason to believe she is pregnant or any woman who is lactating.

3.2.2 Condition that makes MRI unsafe (e.g., cardiac pacemaker, epicardial pacemaker leads, cochlear implants, metal aneurysm clip, metal halo devices)

3.2.3 Inability to tolerate MRI (e.g., unable to lie flat for > 1 hour, severe claustrophobia)

3.2.4 Known allergy to fluorothymidine

3.2.5 Creatinine clearance < 40 ml/min, as estimated by the Cockcroft-Gault formula:

$$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times [0.85 \; if \; Female]}{72 \times \text{Serum Creatinine (in mg/dL)}}$$

3.2.6 Poorly controlled diabetes mellitus (fasting blood glucose > 500 mg/dl)

3.2.7 Institutionalized subject (prisoner or nursing home patient)

3.2.8 Critically ill or medically unstable.

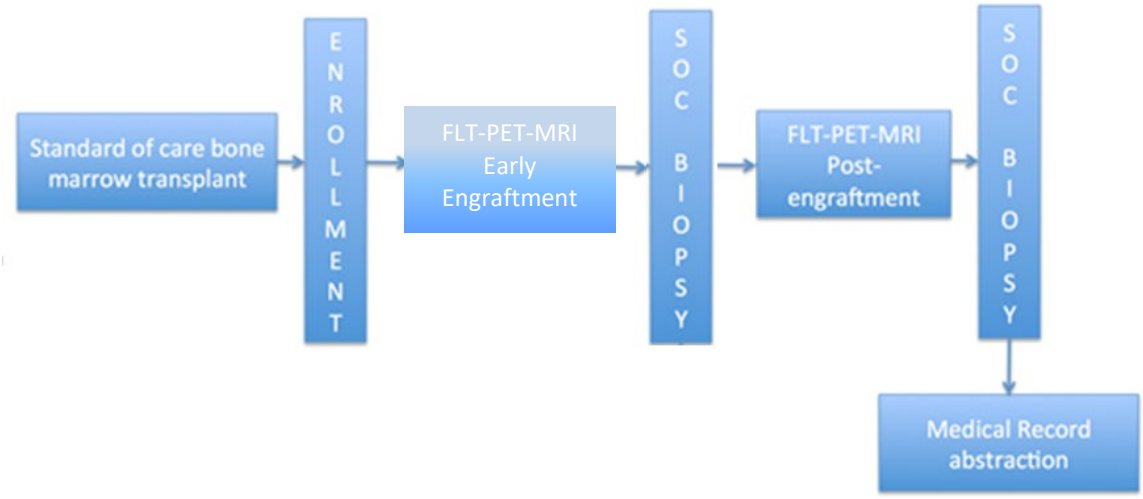
3.2.9 Currently hospitalized (All FLT-PET MRI scans will be obtained in the outpatient setting)

4.0 STUDY PLAN

4.1 Study Duration

It is anticipated that the total study duration encompassing recruitment, enrollment, and data analysis will take approximately 2 years. Active patient participation will last approximately 100 days. Patients will be followed for one year for the development of acute GVHD or graft failure.

4.2 Schema



This is a non-randomized prospective pilot study of patients who have undergone either allogeneic or autologous bone marrow transplants. Subjects will complete FLT-PET/MRI scans on transplant day +25 and on day +60 post-transplant. Only allogeneic transplant patients will undergo restaging marrow biopsies on days +35 and +100 as part of the usual standard of care.

4.3 Enrollment/Recruitment

We will plan to enroll a total of 15 patients in this study. Each patient will be asked to undergo two FLT-PET MRI scans as described above. The study patients who meet the eligibility criteria will be recruited by study personnel in the UNC Bone Marrow Transplant Program.

Once a patient has been referred, the patient will be approached by a coordinator to assess interest in participation. All eligible patients who agree to participate in the study will be asked to come to their scheduled appointment thirty minutes early to complete the informed consent process.

Review of the consent will take place in the privacy of an exam room, or when possible, a sample consent form will be sent to the patient via email prior to the patient's visit to allow for ample review. Once the patient has consented, women of child bearing potential (WCBP) will be given a urine pregnancy test in order to ensure that they are not pregnant.

We are currently seeking to perform a total of 30 FLT-PET MRI scans (two each on 15 patients). If, however, a patient(s) drops out of the study after completing only a single scan, additional patients may be approached for participation.

4.4 FLT-PET/MRI

Experimental measurements will be obtained at two time points: On transplant day +25 +/- 5 days (immediately after neutrophil count recovery) and again on transplant day +60 +/- 5 days (a time point after stable count recovery). FLT PET/MR imaging will be performed. FLT will be obtained from the BRIC Radiochemistry Core. At each FLT-PET-MRI study visit, subjects will complete screening forms pertinent to both PET and MR.

A technologist will attempt to access an existing central venous catheter if appropriately credentialed personnel are on site. If necessary, however, a peripheral IV line will be placed. The patient will then be brought into the scanner room to initiate the scan. FLT (5 mCi) will be injected one hour prior to imaging and dynamic acquisitions will be obtained. Whole body PET/MR will be performed with conventional attenuation correction, whole body STIR (node identification). The MR portion of the scan will be simultaneously obtained *without* the use of gadolinium contrast agent.

4.5 Medical Records Abstraction

Objectives of this study include identifying quantitative imaging markers that best predict engraftment quality and GVHD development in allogeneic HSCT patients. To achieve this aim, we will correlate the imaging data with the need for blood product transfusion support, biopsy based measures of bone marrow donor/recipient chimerism studies and overall bone marrow cellularity, and the clinical and/or histopathological documentation of graft versus host disease.

Patient pathology records will be obtained after the 100-day follow-up period has passed and again one year post-transplant or at the time of death if the patient expires prior to then

All patients receiving HSCT have outcomes data collected for quality and program performance assessments. This data is collected for every patient until either death or lost to follow-up. The data routinely collected includes demographic data, donor match information, and outcomes of engraftment, survival, disease relapse, and development of acute and chronic GVHD.

Image Analysis

Quantitative T1 and DWI ADC maps will be computed. Regions of interests (ROIs) will be drawn on the anatomic MR images (blinded to SUV values and clinical outcome), then transferred to FLT SUV maps. We will correlate the imaging data with the histopathological data to identify the quantitative markers that best predict engraftment quality.

5.0 EXPECTED RISKS/UNANTICIPATED PROBLEMS

5.1 Expected Risks

5.1.1 Risks of PET/MRI

5.1.1.1 IV Placement

For patients, study participation may require placement of an IV for administration of the radioisotope for PET/MRI scans if we are unable to utilize existing central venous access. IV placement may result in pain, bruising or infection. The IV will be placed by a certified BRIC technologist using sterile techniques to minimize the risk of bleeding and infection. Patients will have the option of a topical anesthetic for IV placement to reduce pain and discomfort.

5.1.1.2 Radiation

The FLT-PET/MRI scans will expose subjects to controlled amounts of limited radiation. Subjects will be informed of an estimated dose of radiation as specified by the Radiation Safety Committee. The amount of risk to this estimated dose will be compared to the annual radiation exposure a typical individual receives on a yearly basis from natural background radiation in the informed consent. This radiation exposure involves a small risk and is necessary to obtain the information desired.

Risks arising from the imaging procedure itself including injury to the donor bone marrow and/or additional infectious complications are felt to be extremely low but theoretically possible. Notably FLT-PET scanning has been applied to both the autologous and allogeneic stem cell transplant patient population previously with no reported adverse effects on patient outcomes.[9,11] Furthermore an NCI study is currently ongoing which specifies the use of FLT-PET scanning in

allogeneic stem cell transplants as early as 5 days post-transplant (NCI study NCT01338987, Pilot Study of Lupron to Improve Immune Function After Allogeneic Bone Marrow Transplantation). Nevertheless, the following assessments will be undertaken to carefully monitor for any unexpected effects on peripheral blood counts or susceptibility to infection.

5.1.1.2.1 Allogeneic Transplant Patients

All allogeneic transplant recipients on study will undergo at least twice weekly complete blood count analyses and complete metabolic panels (i.e. blood chemistry including electrolytes, serum creatinine and BUN, liver function tests) during the first 90 days post-transplant. All allogeneic transplant recipients on study will also undergo weekly quantitative viral loads for CMV and EBV. Additional infectious testing will be obtained as clinically indicated. Notably, all of these evaluations are already obtained for all allogeneic stem cell transplant patients at The University of North Carolina as part of the standard of care. It is routine practice at UNC for all allogeneic transplant recipients to be seen 2-3 times weekly in the bone marrow transplant clinic over the entire first 100 days post-transplant.

5.1.1.2.2 Autologous Transplant Patients

Autologous transplant patients on study will undergo at least two complete blood count analyses and two complete metabolic panels during the week immediately following their first scan, and at least one CBC and one comprehensive metabolic panel following their second scan. Since the second scan will be obtained many weeks after count recovery, any risk to the donor bone marrow product is considered to be even less at this time point, and hence only a single set of labs is mandated. As CMV and EBV reactivation are extremely rare in the autologous transplant patients, viral loads for these agents will not be mandated. Rather, any relevant infectious work-up will be guided by the clinical circumstances.

5.1.1.3 FLT

FLT will be available through the Biomedical Research Imaging Center Radiopharmaceutical Laboratory as an investigational drug under and National Cancer Institute NCI IND. The associated risks and drug details are listed in Appendix A.

5.1.2 Patient Confidentiality

The risk of breach of confidentiality is low and will be minimized by securing all identifiable data in locked cabinets and password-protected electronic medical records. Imaging data will be de-identified during acquisition, labeled with a study ID and post-processed on secure computers.

6.0 TIME AND EVENTS TABLE

6.1 Time and Events Table

	Baseline	+25 after BMT +/- 5 days	35 days after BMT +/- 5 days	+60 days after BMT +/- 5 days	100 days after BMT +/- 5 days
Screening	X				
Informed Consent	X				
FLT-PET-MR		X		X	
Standard of Care Bone Marrow Biopsy[a]			X		X
	[a] Allogeneic transplant patients only				

7.0 ADVERSE EVENTS

7.1 Definition

7.1.1 Adverse Event (AE)

An adverse event (AE) is any untoward medical occurrence (e.g., an abnormal laboratory finding, symptom, or disease temporally associated with the use of a medicinal product, in this case the FLT radiotracer) in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) need not be considered AEs and should not be recorded as an AE. Disease progression should not be recorded as an AE, unless it is attributable by the investigator to the study therapy.

As defined by UNC's IRB, unanticipated problems involving risks to study subjects or others (UPIRSO) refers to any incident, experience, or outcome that:

- Is unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent

document; and (b) the characteristics of the subject population being studied;
- Is related or possibly related to a subject's participation in the research; and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

7.1.2 A suspected adverse reaction (SAR) is any AE for which there is a *reasonable possibility* that the drug/radiotracer is the cause. *Reasonable possibility* means that there is evidence to suggest a causal relationship between the drug/radiotracer and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug/radiotracer.

Causality assessment to a study drug/radiotracer is a medical judgment made in consideration of the following factors: temporal relationship of the AE to study drug/radiotracer exposure, known mechanism of action or side effect profile of study treatment, other recent or concomitant drug exposures, normal clinical course of the disease under investigation, and any other underlying or concurrent medical conditions. Other factors to consider in considering drug/radiotracer as the cause of the AE:

- Single occurrence of an uncommon event known to be strongly associated with drug/radiotracer exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome)
- One or more occurrences of an event not commonly associated with drug/radiotracer exposure, but otherwise uncommon in the population (e.g., tendon rupture); often more than once occurrence from one or multiple studies would be needed before the investigator could determine that there is *reasonable possibility* that the drug/radiotracer caused the event.
- An aggregate analysis of specific events observed in a clinical trial that indicates the events occur more frequently in the treatment group than in a concurrent or historical control group

7.1.3 Unexpected AE or SAR

An AE or SAR is considered <u>unexpected if</u> the specificity or severity of it is not consistent with the applicable product information (e.g., Investigator's Brochure (IB) for an unapproved investigational product or package insert/summary of product characteristics for an approved product). Unexpected also refers to AEs or SARs that are mentioned in the IB as occurring with a class of drugs/radiotracers or as anticipated from the pharmacological properties of the drug/radiotracer, but are not specifically mentioned as occurring with the particular agent under investigation.

7.1.4 Serious AE or SAR

An AE or SAR is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death;
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- Requires inpatient hospitalization (>24 hours) or prolongation of existing hospitalization;*
- Results in congenital anomaly/birth defect;
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. For reporting purposes, also consider the occurrences of pregnancy as an event which must be reported as an important medical event.

*Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.

Pregnancy that occurs during the study must also be reported as an SAE.

7.2 Documentation of non-serious AEs or SARs

For non-serious AEs or SARs, documentation must begin from day 1 of study treatment and continue through the 30 day follow-up period after treatment is discontinued, in this case the second planned FLT-PET MRI scan.

Collected information should be recorded in the Case Report Forms (CRF) for that patient. Please include a description of the event, its severity or toxicity grade, onset and resolved dates (if applicable), and the relationship to the study drug. Documentation should occur at least monthly.

7.3 SAEs or Serious SARs

7.3.1 Timing

After informed consent but prior to initiation of study medications/radiotracer administration, only SAEs caused by a protocol-mandated intervention will be collected (e.g. SAEs related to invasive procedures such as biopsies, medication washout).

For any other experience or condition that meets the definition of an SAE or a serious SAR, recording of the event must begin from day 1 of study treatment and continue through the 30 day follow-up period after treatment is discontinued, in this case the second planned FLT-PET MRI scan.

7.3.2 Documentation and Notification

SAEs or Serious SARs must be recorded in the SAE console within Oncore™ for that patient within 24 hours of learning of its occurrence. Additionally, the NCCN Project Manager must also be notified via email of all SAEs within 24 hours of learning of its occurrence.

7.3.3 Reporting

IRB Reporting Requirements:

UNC**:**

UNC will submit an aggregated list of all SAEs to the UNC IRB annually at the time of study renewal according to the UNC IRB policies and procedures.

The UNC-IRB will be notified of all SAEs that qualify as an Unanticipated Problem as per the UNC IRB Policies using the IRB's web-based reporting system (see section 9.5.3) within 7 days of the Investigator becoming aware of the problem.

Pregnancy

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on study should be recorded as SAEs. The patient is to be discontinued immediately from the study. The female subject should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.

The Investigator will follow the female subject until completion of the pregnancy, and must document the outcome of the pregnancy (either normal or abnormal outcome) and report the condition of the fetus or newborn to the UCCN Project Manager. If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE.

FDA Expedited Reporting requirements for studies conducted under an IND:
If an investigator deems that an event is both a serious SAR AND unexpected, it must also (in addition to Oncore) be recorded on the MedWatch Form 3500A as per 21 CFR 312.32. Unexpected adverse events or adverse reaction refers to an event or reaction that is not listed in the investigator's brochure or is not listed at the specificity or severity that has been observed; or if an investigator's brochure is not

required or available, is not consistent with the risk information described in the general investigation plan or elsewhere in the current IND application.

The MedWatch form should be faxed to the UNCCN Project Manager at 919-966-4300 (or emailed, with address provided at the Start up Meeting (SIM)) along with supporting documentation defining the event and causality. The UNCCN Project Manager will then send the report to the Funding Source.

Once the UNC Principal Investigator determines an event is a serious SAR AND unexpected, the MedWatch 3500A form will be submitted to the FDA by the UNCCN Project Manager. If the event is serious, unexpected and considered to be possibly-, probably- or definitely-related to the study treatment, the UCCN Project Manager will inform the Regulatory Associate at UNC who will be responsible for submitting the SAR to the IND. All IND safety reports must be submitted on Form 3500A and be accompanied by Form 1571. The FDA must be notified or any unexpected or life-threatening suspected adverse reactions as soon as possible, but no later than 7 calendar days of learning of the event.

The UNCCN Project Manager will also be responsible for informing each Affiliate site of all serious and unexpected SARs reported to the FDA via fax as soon as possible.

7.4 Data and Safety Monitoring Plan

The Principal Investigator will provide continuous monitoring of patient safety in this trial.

Meetings/teleconferences will be held at a frequency dependent on study accrual, and in consultation with the study Biostatistician. These meetings will include the investigators as well as protocol nurses, clinical research associates, regulatory associates, data managers, biostatisticians, and any other relevant personnel the principal investigators may deem appropriate. At these meetings, the research team will discuss all issues relevant to study progress, including enrollment, safety, regulatory, data collection, etc.

The team will produce summaries or minutes of these meetings. These summaries will be available for inspection when requested by any of the regulatory bodies charged with the safety of human subjects and the integrity of data including, but not limited to, the oversight (Office of Human Research Ethics (OHRE) Biomedical IRB, the Oncology Protocol Review Committee (PRC) or the North Carolina TraCS Institute Data and Safety Monitoring Board (DSMB).

The UNC LCCC Data and Safety Monitoring Committee (DSMC) will review the study on a regular (quarterly to annually) basis, with the frequency of review based on risk and complexity as determined by the UNC Protocol Review Committee. The UNC PI will be responsible for submitting the following

information for review: 1) safety and accrual data including the number of patients treated; 2) significant developments reported in the literature that may affect the safety of participants or the ethics of the study; 3) preliminary response data; and 4) summaries of team meetings that have occurred since the last report. Findings of the DSMC review will be disseminated by memo to the UNC PI, PRC, and the UNC IRB and DSMB.

8.0 STATISTICAL CONSIDERATIONS

8.1 Study Design

This is a non-randomized, prospective pilot study exploring the use of FLT-PET-MRI in the evaluation of allogeneic patients after bone marrow transplants to potentially predict engraftment success and the development of graft versus host disease. A total of 15 patients who have undergone bone marrow transplantation will receive FLT-PET-MRI imaging, 3 autologous and 12 allogenic recipients will be recruited. The 3 autologous patients are included as a comparator arm to help elucidate how much of the FLT signal in allogeneic HSCT patients is related to allo-antigen driven T cell proliferation and therefore unique to the allogeneic setting.

8.2 Sample Size and Accrual

This is a pilot study to gather preliminary data, and the results from this study will be used to power a future larger R01. However, we will have power to detect large differences between groups as follows. For power calculation, the null hypothesis is that there will be no difference in the FLT bone marrow signal between those allograft patients demonstrating incomplete and complete donor reconstitution on their restaging marrow biopsies, and no difference in the FLT signal within secondary lymphoid tissues between patients with and without acute GVHD.

<u>Complete Donor Reconstitution:</u>
Considering only the 12 allogeneic patients, we will compare the mean FLT bone marrow signal between those with and without complete donor reconstitution. In general we would expect approximately 50-70% of our patients overall to achieve complete donor reconstitution in both the unfractionated and CD3 enriched compartments by transplant day +30.

Assuming equal number of patients with/without Complete Donor Reconstitution:

A sample size of 6 in each group will have 80% power to detect a probability of 0.854 that an observation in the Complete Donor Reconstitution group is less than an observation in the non-Complete Donor Reconstitution group using a Wilcoxon (Mann-Whitney) rank-sum test with a 0.100 one-sided significance level.

Assuming unequal number of patients with/without Complete Donor Reconstitution:

A total sample size of 12 will have 80% power to detect a probability of 0.87 that an observation in the Complete Donor Reconstitution group (n=7) is less than an observation in the non-Complete Donor Reconstitution group (n=5) using a Wilcoxon (Mann-Whitney) rank-sum test with a 0.100 one-sided significance level.

Graft Versus Host Disease:
Considering only the 12 allogeneic patients, we will compare the mean FLT signal within secondary lymphoid tissues in those patients who develop acute GVHD versus those who do not based on the criteria described previously. In general we would expect that approximately 30-40% of our patients will develop some degree of acute GVHD (any site and any clinical grade) by transplant day +100

A sample size of 6 in each group will have 80% power to detect a probability of 0.854 that an observation in the non-GVHD group is less than an observation in the GVHD group using a Wilcoxon (Mann-Whitney) rank-sum test with a 0.100 one-sided significance level.

8.3 Data Analysis Plans

8.3.1 Primary Analysis

Experimental measurements will be obtained at two time points: around day +21 (at the time of initial neutrophil engraftment) and around day +60 (after stable count recovery). FLT standardized uptake value (SUV) will be evaluated within the following target organs: The marrow signal will be evaluated in the pelvis, vertebrae, and bilateral femurs. The secondary lymphoid tissue signal will be evaluated in the spleen, inguinal lymph nodes, axillary lymph nodes, cervical lymph nodes, and mesenteric lymph nodes). We will plan to image 3 autograft and 12 allograft patients.

Wilcoxon Rank sum tests will be used to compare signals between the groups. Spearman and Pearson correlation coefficients will be used to measure correlations between pairs of continuous variables. ROC analyses will be performed to explore the sensitivity/specificity of signal ratio cutoffs for the outcomes of Complete Donor Reconstitution and GVHD.

8.4 Data Management/Audit

The images of all eligible enrolled subjects that are obtained will be de-identified for inclusion in the reader study. Copies of the clinical report forms as well as the

de-identified images described in the preceding will be submitted for each case to the Study Coordinators for maintaining the study record and entering the data into a spreadsheet in preparation for the reader study.

As an investigator initiated study, this trial may also be audited by the Lineberger Cancer Center audit committee every twelve months.

9.0 STUDY MANAGEMENT

9.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

9.2 Required Documentation

Before the study can be initiated at any site, the following documentation must be provided to the Clinical Protocol Office (CPO) at the University of North Carolina.

- A copy of the official IRB approval letter for the protocol and informed consent
- CVs and medical licensure for the principal investigator and any associate investigators who will be involved in the study
- A copy of the IRB-approved consent form

9.3 Registration Procedures

Patients will be registered into OnCore®, a web based clinical research platform by one of the Study Coordinators. The spread sheet contains each subject enrolled in the study identified by the patient first and last initial, study id, date of enrollment into study, race and ethnicity.

9.4 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

9.4.1 Emergency Modifications

UNC investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior UNC IRB approval.

For any such emergency modification implemented, a UNC IRB modification form must be completed by UNC Research Personnel within five (5) business days of making the change.

9.4.2 Single Patient/Subject Exceptions

Any request to enroll a single subject who does not meet all the eligibility criteria of this study requires the approval of the UNC Principal Investigator and the UNC IRB.

9.4.3 Other Protocol Deviations/Violations

According to UNC's IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

An unplanned protocol variance is considered a violation if the variance meets any of the following criteria:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

If a deviation or violation occurs please follow the guidelines below:

Protocol Deviations: UNC personnel will record the deviation in OnCore® (or other appropriate database set up for the study), and report to any sponsor or data and safety monitoring committee in accordance with their policies. Deviations should be summarized and reported to the IRB at the time of continuing review.

Protocol Violations: Violations should be reported by UNC personnel within one (1) week of the investigator becoming aware of the event using the same IRB online mechanism used to report UPIRSO.

Unanticipated Problems Involving Risks to Subjects or Others (UPIRSO: Any events that meet the criteria for "Unanticipated Problems" as defined by UNC's IRB (see section 6.1) must be reported by the Study Coordinator using the IRB's web-based reporting system.

- Has damaged the scientific integrity of the data collected for the study.

9.5 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to UNC's IRB for approval prior to implementation.

9.6 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

9.7 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

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11.0 APPENDICES

Appendix A: FLT

1.1 Chemical Name

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

1.2 Pharmacology and Toxicology

FLT is a structural analog of the DNA constituent, thymidine. Thymidine has previously been labeled with C-11 for studies in cell culture and animals; results have shown that it is rapidly incorporated into newly synthesized DNA.[38] Radiolabelled thymidine has been used for noninvasive evaluation of tumor proliferation. Shields *et al* determined that [C-11] thymidine demonstrated a response to chemotherapy faster than ^{18}F-FDG in 6 patients with malignancy.[38] However, because C-11 has a half-life of 20 minutes,[38] it is not practical for routine clinical use and other radiolabels are being investigated. ^{18}F-FLT is radiolabelled with F-18, which has a half-life of 110 minutes.[38]

The pharmacology of FLT is based on its action as an inhibitor of DNA synthesis.[41-43] Intracellular metabolism of FLT produces nucleotides that inhibit endogenous DNA polymerases because they lack a 3'-hydroxyl substituent. This results in premature chain termination of DNA synthesis.[44, 45] These biochemical properties can account for FLT's prominent hematological and liver toxicity.[45-47] The pharmacology of FLT closely parallels that of the widely used prescription HIV-antiviral drug azidothymidine (AZT).[48, 49] Both FLT and AZT are 3'-deoxythymidine analogs that act as inhibitors of DNA synthesis and are cleared from the body in the same way. However, FLT is significantly more cytotoxic than AZT in test cell lines.[47] Cellular uptake of FLT and thymidine is greater than that of AZT. Transport of FLT and thymidine across cell membranes occurs by active transport and passive diffusion.[49]

1.3 Toxicity of FLT in Humans

FLT was investigated as an anti-AIDS drug in humans.[46] Toxic effects and death were reported for some subjects who received FLT during randomized concentration-controlled trials during a 16-week treatment of oral multi-dosing. Doses of 0.125 mg/Kg every 12h produced a mean cumulated drug exposure (AUC12: area under curve) of 417 ng-h/mL. At this level, serious (grade 3) hematologic toxicity occurred in 6 of 10 subjects. At 300 ng-h/mL, grade 2 or greater (fall in hemoglobin to < 9.4 g/dL) developed within 4 weeks in 9 of 12 subjects. At 200 ng-h/mL almost no clinically significant anemia developed, but dose-limiting granulocytopenia (< 750 granulocytes/mm^3) occurred in 5 of 15 subjects. Mild peripheral neuropathy occurred in 2 of 10 subjects at 50 ng-h/mL, but was not dose-limiting. FLT drug trials were terminated after the unexpected death of 2 subjects of hepatic failure. One patient assigned to 200 ng-h/mL developed progressive liver failure and died after 12 weeks of FLT therapy. A second subject, who received a fixed dose of 10 mg/day, developed progressive liver failure and died at about the same time. All surviving subjects were followed closely for 4 weeks after stopping FLT and none had evidence of clinically significant liver disease or other adverse effects. Overall, 25 of the 44 subjects receiving at

least two doses of FLT completed the 16 week study without clinically significant adverse effects.

Unlabelled FLT was initially investigated as a treatment for HIV and AIDS, and toxicity studies of the unlabeled compound have been performed at substantially higher doses than those proposed for imaging. Hematologic, hepatic and peripheral nerve toxicities were observed after administration of therapeutic doses (≥ 10 mg) of FLT for several weeks (See Section 9.1.10). In comparison, the proposed ^{18}F-FLT PET studies use a maximum injection of 10μg, a factor of 1,000 times lower[50]. The dose of FLT to be administered in this imaging trial is 1400-fold lower than the dose that led to serious toxicity in the studies described above.

1.4 Dosimetry

An [^{18}F]FLT dose of 0.07 mCi/Kg with a maximum of 5 mCi was selected based on a prior human dosimetry study performed in 18 patients at the University of Washington[51]. With this dose, the individual organ and total-body radiation dose associated with [^{18}F]FLT is comparable to or lower than those reported for widely used clinical nuclear medicine procedures. There is ample preliminary evidence that a dose of 5 mCi is sufficient for imaging. The actual dosing, 0.07 mCi/kg was determined by assuming average body weight of 70 Kg and dividing by the maximum total dose. As FLT is not lipid soluble, no upward adjustments are expected to be needed for subjects > 70 Kg. These details are specified in the IND. A summary of the relevant human dosimetry for 2 different voiding scenarios from the investigator's brochure is included in Table 1. For more details, the reader is referred to the IND.

Table 1. Human dosimetry estimates

Organ of Interest	Men mGy/MBq (mrad/mCi)	Women mGy/MBq (mrad/mCi)
Total Body Dose	Scenario 1 1.23E-02 (46) Scenario 2 1.26 E-02 (47)	Scenario 1 1.56E-02 (58) Scenario 2 1.59 E-02 (59)
Bladder	Scenario 1 1.79E-01 (662) Scenario 2 7.91E-02 (293)	Scenario 1 1.74E-01 (646) Scenario 2 7.76E-02 (287)
Liver	Scenario 1 4.51E-02 (167) Scenario 2 4.54 E-02 (168)	Scenario 1 6.42E-02 (238) Scenario 2 6.45 E-02 (239)

Scenario 1: Single bladder voiding at 6 h after [^{18}F]FLT administration with a 10% post-voiding bladder residual decayed to infinity. This scenario assumed no urine re-accumulation after 6 h.
Scenario 2: First bladder voiding at 2 h after [^{18}F]FLT administration with a 10% post-voiding residual; urine re-accumulation between 2 and 6 h at a rate determined by the bladder curve fit; second bladder voiding at 6 h with a 10% post-voiding residual decayed to infinity. This scenario assumed no urine re-accumulation after 6 h.
The first scenario is conservative, whereas the second has a more realistic voiding scheme.

1.5 Previous human [^{18}F]FLT imaging studies

Several preliminary studies using [^{18}F]FLT imaging in human subjects have been performed in Germany and the United States (UCLA, University of Washington in Seattle, Wayne State University)[51-56]. The imaging protocols were pre-approved by their respective regulatory committees and conducted under the RDRC process, with patients receiving between 1.4 and 13 mCi of [^{18}F]FLT. The group in Seattle, which has the most experience with this agent in the US, has performed numerous studies in patients with lung cancer as well as a few in patients with primary brain tumors. Their findings demonstrate the feasibility and merit of tumor imaging with [^{18}F]FLT. [^{18}F]FLT PET showed increased uptake in tumor lesions outside the liver or bone marrow with standardized uptake values (SUV) of 4-7, enabling differentiation from surrounding tissues (SUV 0.5-2).

1.6 Reported Adverse Events and Potential Risks

No adverse events have been reported for [^{18}F]FLT at the dose to be used for this study. As described in section 6.2, non-radioactive FLT has been investigated as an anti-AIDS drug, and some adverse effects, namely, reversible peripheral neuropathy, were observed in subjects exposed to 50 ng-h/mL plasma over a course of 16 weeks (15μg/kg q12h). The FLT dose anticipated for this study will be <6.1μg for a single injection. Assuming a 70kg individual, the maximum concentration of FLT would be expected to be equivalent to 0.29 ng-h/mL. The radiation exposure associated with this study is described in section 3.3 and is comparable to the dose for other widely used clinical nuclear medicine procedures.

1.7 [^{18}F]FLT Administered Dose

The administered dose will be 0.07 mCi/kg with a maximum of 5 mCi. The drug solution is stored at room temperature in a gray butyl septum sealed, sterile, pyrogen-free glass vial and has an expiration time of 8 hours. The injectable dose of [^{18}F]FLT for most studies will be ≤ 0.07 mCi/kg of fluorine-18, not to exceed 5 mCi with a specific activity greater than 200 Ci/mmol at the time of injection. 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. [^{18}F]FLT is administered to subjects by intravenous injection of ≤ 10 mL. There is no evidence that nonradioactive and radioactive FLT molecules display different biochemical behavior.

1.8 Agent Availability

[^{18}F]FLT will be provided by the Biomedical Research Imaging Radiopharmaceutical Core under an IND held by the Cancer Imaging Program (CIP)/NCI. The cross reference letter has been obtained.

Appendix B: Reader Study Data Collection Form

Patient ID: _____ **Reader:_____**
Date:_____

Overall Assessment:

FLT SUV in pelvis _____

FLT SUV in vertebrae _____

FLT SUV in right femur _____

FLT SUV in left femur _____

FLT SUV in inguinal lymph nodes _____

FLT SUV in axillary lymph nodes _____

FLT SUV in cervical lymph nodes _____

FLT SUV in mesenteric lymph nodes _____