First in-human PET imaging studies with NIS reporter $[^{18}F]BF_4$

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IRB Protocol

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1. INTRODUCTION

With the explosive growth of gene and cell-based therapies, there remains a large unmet clinical need for sensitive and quantitative imaging methods for reporter gene monitoring. Radionuclide-based techniques offer the highest sensitivity for monitoring reporter expression, with PET offering the most promise. A number of reporter systems employing PET probes have been described, all with their own strengths and weaknesses. The ideal reporter probe would have negligible uptake in all normal tissues, with rapid excretion through the kidneys to allow excellent target:background contrast in transduced tissues. None of the probes developed to date exhibit this ideal behavior; each have uptake in one or more normal tissues that would obscure imaging of gene transduction in those tissues. Endogenous sodium/iodine symporter (NIS) expression is high in thyroid,salivary glands, and stomach. These organs would be problematic for reporter imaging. However, there is low expression of NIS in liver, spleen, kidney, skeletal muscle and other organs which can be problematic for the other reporter systems. Regarding imaging sensitivity, the probes that rely on a transport or metabolic trapping mechanism (tk, NIS, NET) tend to show higher tissue:blood ratios than probe systems that are based on receptor binding (D2R, somatostatin R). Based on these properties, we believe monitoring of NIS transduction using [^{18}F]BF_4-PET may have distinct advantages in applications that involve gene therapy in liver, spleen, kidneys, bone marrow and skeletal muscle. Furthermore, since NIS is a self-protein (unlike HSV-tk), it minimizes the possibility of immune-mediated destruction of cells encoding the reporter. On the other hand, NIS will not be desirable as a reporter in thyroid, stomach or bladder.

The NIS reporter system has unique properties for monitoring gene transduction. A number of molecules besides radioiodide can be used as imaging probes for NIS, including the SPECT isotope [^{99m}Tc]pertechnetate. Recently, [^{18}F]BF_4 has been revisited as a promising NIS imaging agent for PET [1, 2]. Jauregui-Osoro et al. [1] updated the synthesis with newer materials that have become available commercially, but the method remained isotopic exchange. These researchers expected that a maximum specific activity that can be achieved with current ^{18}F fluoride production levels is ~1GBq/μmol. Since NIS is a saturable transporter and ultimately the “shelf-life” of a distributed PET probe could be several hours, it is highly desirable that a high specific activity synthesis of [^{18}F]BF_4 be developed. We have developed a practical, high-yield method for production of high specific activity [^{18}F]BF_4.

We have performed preclinical imaging studies to confirm the high avidity uptake of [^{18}F]BF_4 using a C6 glioma mouse tumor model. hNIS-transfected C6 glioma cells were implanted subcutaneously in the flank of athymic nude mice and allowed to grow to ~5 mm diameter before imaging. Dynamic PET imaging was performed in hNIS-expressing C6 glioma xenografted mice following retro-orbital or IP injection ~1.1 MBq Na [^{18}F]BF_4 (Figure 1). [^{18}F]BF4 showed highly specific uptake in NIS expressing tissues: thyroid, stomach, salivary glands and mammary glands. This distribution exactly matches the well-known normal distribution of radioiodides. Urinary excretion was variable. Uptake increased in tumor over 1 hr. From biodistribution data, tracer uptake at 60 min p.i. was >300 fold higher in C6 tumor expressing NIS relative to control (NIS negative) tumor. Uptake of tracer increased in NIS expressing tumor and thyroid with increasing specific activity (Figure 1E). These preliminary data corroborate previously published findings and our recent imaging with [^{18}F]BF4 in mice bearing osteosarcoma tumors (3) that show that the tetrafluoroborate anion serves as an excellent iodide analog for NIS-mediated transport and that [^{18}F]BF4 may serve as a highly specific and reliable PET reporter probe for NIS activity in tissues. They strongly support the translation of [^{18}F]BF4 for clinical imaging. The improvement in specific activity with our newly developed synthesis method will also compensate for prolonged distribution times of the radiotracer in a commercial distribution setting.

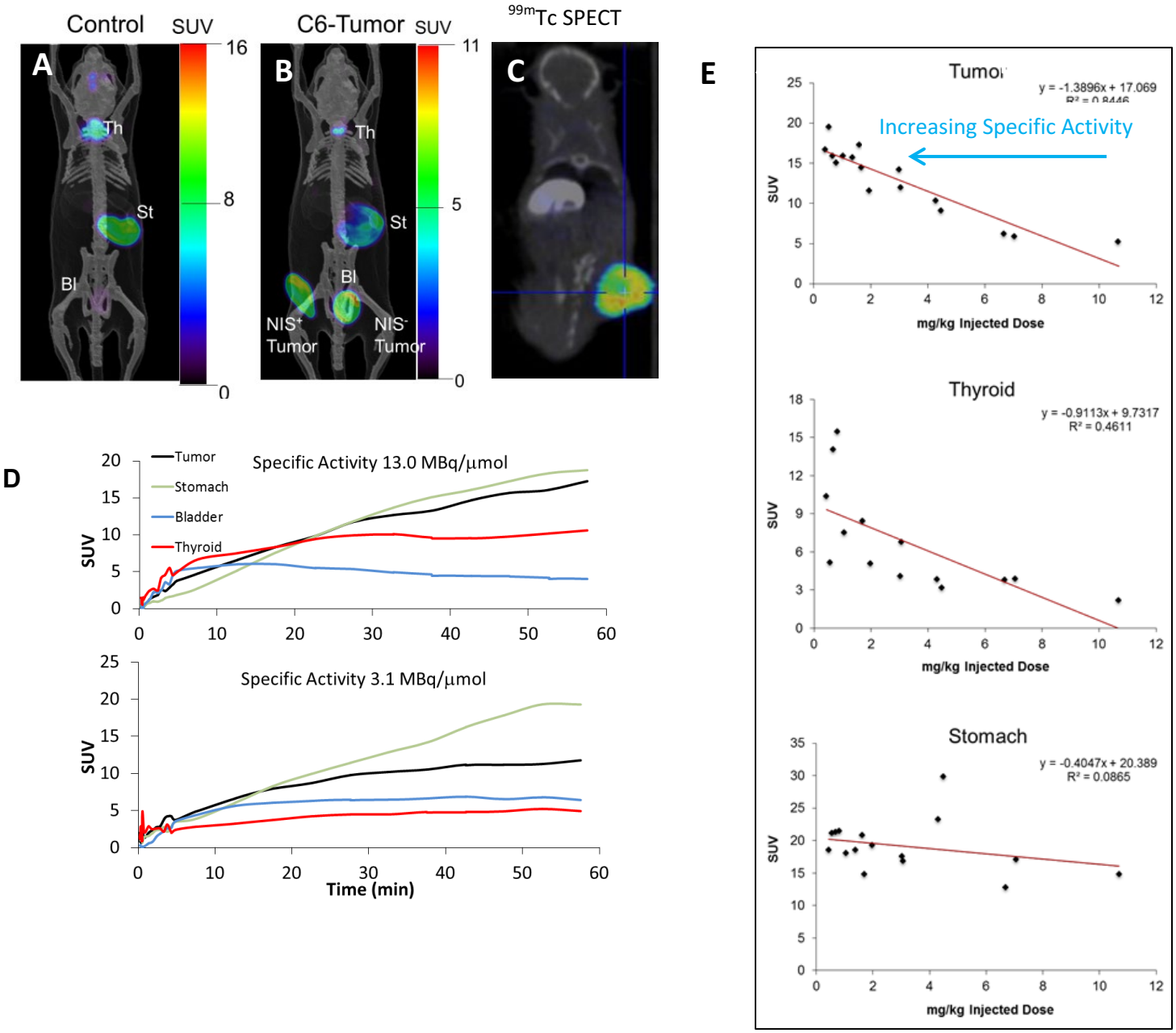

Figure 1. [^{18}F]BF_4 PET images in (A) normal athymic nude mouse and (B) athymic nude mouse bearing hNIS expressing C6 glioma xenograft. Th-Thyroid, St-Stomach, and Bl-Bladder. Opposite tumor not expressing hNIS showed insignificant tracer uptake. (C) ^{99m}Tc SPECT image of athymic nude mouse bearing hNIS expressing C6 glioma xenograft.(D) Time-activity curves in tumor, stomach, thyroid and bladder for different specific activity (SA) preparations of [^{18}F]BF_4. Tumor uptake is decreased with decreasing SA. (E) Effect of specific activity on [^{18}F]BF_4 uptake in tumor, thyroid and stomach.

With the advent of the high specific activity synthesis method and confirmation of the beneficial imaging properties of [^{18}F]BF_4 in our own preclinical studies in a NIS-expressing C6 tumor mouse model, we believe that [^{18}F]BF4 is ready to be tested for imaging feasibility in human patients. We therefore propose the first in-human studies with [^{18}F]BF_4 in normal subjects (for biodistribution, metabolism, and dosimetry data), and patients with myelomas undergoing experimental treatment with Measles virus (MV) containing NIS as a reporter. ^{99m}Tc-SPECT scans will be performed in the same patients to provide a comparison of imaging sensitivity for the two modalities. This study will provide essential data on imaging characteristics of [^{18}F]BF_4 in human patients and provide an initial assessment of detection sensitivity for NIS activity for monitoring transduction efficacy of oncoviral therapy.

2. STUDY OBJECTIVES

We propose to develop ^{18}F-labeled sodium tetrafluoroborate ([^{18}F]BF_4) as a more sensitive and quantitative probe of NIS activity in tumors. We currently produce [^{18}F]BF4 at the Mayo Clinic and have demonstrated initial promising results in animal studies. This clinical trial is designed to test if [^{18}F]BF_4 can detect NIS activity in tumors under conditions that would substantially improve cancer therapy by: 1) assessing evidence of viral infection and 2) assessing the durability of viral replication in tumor by monitoring NIS uptake at baseline (before virus treatment) and day +9. [^{18}F]BF_4 –PET will be compared with conventional SPECT imaging to determine the relative advantage for NIS activity detection and quantification in tumors. Since [^{18}F]BF_4 has not been used in human studies to our knowledge, we will establish fundamental pharmacokinetic, biodistribution and radiation dosimetry data for the imaging drug. This data will be necessary to support future regulatory submissions. The specific aims of the R01 proposal submitted to NIH are:

Aim 1. To develop an automated synthesis of high-specific activity [^{18}F]BF_4 and establish standard operating procedures and documentation for human applications. (This aim concerns radiochemistry development and does not involve human subjects, so is not relevant to this IRB application. We have not renumbered Aims 2 and 3 to provide consistency between the IRB and the NIH application.)

Aim 2. Define the fundamental biodistribution, metabolism and radiation dosimetry characteristics for [^{18}F]BF_4 in human subjects. Dynamic trunk [^{18}F]BF_4-PET imaging will be performed in 8 normal adult human subjects (4 male, 4 female) over a 4 h period. IV blood samples will be taken and analyzed to determine radiochemical stability. Dosimetry estimates will be generated from the regional uptake data. Normal NIS-mediated activity in thyroid, stomach and salivary glands will be quantified by parametric (compartmental modeling) and nonparametric (SUV) methods.

Aim 3. Perform [^{18}F]BF_4-PET imaging in 10 patients with myeloma before MV-NIS treatment and 10 patients with endometrial cancer before VSV-hINFβ-NIS treatment, and at 9 d following therapy to monitor NIS activity in the tumors. To show the correlation of positive regional uptake with tissue histopathology for NIS, when accessible, biopsies will be taken after the day 9 scan.

NOTE: Tc-99m SPECT scans will be performed for comparison purposes under separate IRB approvals (06-005263 and 15-007000) and are not included in this protocol.

3. SPONSOR, INVESTIGATOR(S) AND OTHER PARTICIPANTS

The study is sponsored by:

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One center in the United States will participate.

4. TEST DRUG AND CONTROL AGENTS

4.1 Descriptive Name: Na [^{18}F]BF_4

Sodium [^{18}F]BF_4

$NaBF_4$

MW = 109.8 amu

4.2 Radioactive Labeling

The compound is labeled with fluorine-18 [^{18}F] that decays by positron (β^+) emission and has a half-life of 109.8 min. The principal photons useful for diagnostic imaging are the 511 keV gamma photons, resulting from the interaction of the emitted positron with an electron.

4.3 Decay Characteristics

The time course of radioactive decay for Fluorine-18 [^{18}F] is shown below in Table 1.

Table 1: Physical decay chart for Fluorine-18 [^{18}F]. Half -life = 109.8 min.

Min.	Fraction Remaining
0	1.000
20	0.8814
40	0.7768
60	0.6847
80	0.6035
100	0.5319
120	0.4688
150	0.3879
180	0.3210

4.4 Synthesis of Compound Na [^{18}F]BF_4 .

Synthesis of [^{18}F]BF_4 will be performed at the Mayo Clinic Cyclotron Facility in Charlton North building by Dr. Huailei Jiang under the supervision of the PI. An automated synthesis of [^{18}F]BF_4 has been developed whereby cyclotron-produced [^{18}F]fluoride is trapped on a quaternary methyl ammonium anion exchange (QMA) cartridge, then allowed to react with BF_3 freshly pre-formulated in a petroleum ether and tetrahydrofuran (THF) (50 : 1) solution. The resultant [^{18}F]BF_4 product is retained on the QMA cartridge. The cartridge is washed with THF and water to remove unreacted BF_3 and residual solvent. The product [^{18}F]BF_4 is eluted from the cartridge using isotonic saline solution. The final product is passed through two neutral alumina cartridges before final sterile filtration to remove unreacted fluoride. The final radiochemical purity and identity of the drug is determined by radiometric ion chromatography HPLC. Radiochemical purity must exceed 95% in order to inject. Other quality control (QC) tests will include appearance, radionuclidic identity, radiochemical identity (rHPLC), chemical purity (HPLC-CD), radionuclidic purity (HPGe detector), specific activity, pH, residual solvents (GC-FID), sterility and pyrogens. The criteria for passing the QC tests are shown in Table 3. All tests

must be passed for release of the product to be administered to the research subjects. Three consecutive validation syntheses were performed in Feb-Mar, 2016, all passing the designated QC tests.

Table 2. Quality control tests and acceptance criteria for Na [^{18}F]BF_4

Test	Acceptance Criteria		Test	Acceptance Criteria
Appearance	Colorless and free from particulate matter		Specific activity	> 0.5 GBq/μmol
Radionuclidic identity	Half-life is 109.8 ± 5.5 min		Filter integrity	> 50 psi
Radiochemical identity	HPLC: Retention time (R_t) of F-18 BF_4 = ± 10% retention time of TFB std		Endotoxins	< 175 EU/ V^3
Radiochemical purity	HPLC: Activity of F-18 TFB peak ≥ 95% total radioactivity		Sterility (2 wks later; not used for product release)	Pass
Radionuclidic purity[4]	≥ 99.5 % of the observed peaks should correspond to 0.511 MeV, 1.022 MeV, or Compton scatter peaks of [^{18}F]			
pH	4.5 – 7.5			
Residual solvent : Acetone Petroleum Ether THF	≤ 0.50 % (w/v) ≤ 0.03 % (w/v) ≤ 0.072 % (w/v)			
Radioactive Concentration	1–100 mCi/mL @ EOS			
Osmolality (Isotonicity) Calculated[4]	250-359 mOsmoles/L			

4.5 Formulation and Dose

The drug is formulated in a 0.9% sodium chloride, USP injection. [^{18}F]BF_4 will be administered i.v. in a tracer amount of 9-11 mCi. The expected specific activity of [^{18}F]BF_4 is 1-10 GBq/μmol. We have set the acceptance criterion for specific activity as greater than 1 GBq/μmol. At a 9-11 mCi (333-407 MBq) human dose, the mass of [^{18}F]BF_4 will be 0.33-0.41 μmol or 36-44 μg. Higher doses may be administered to correct for the radioactive decay of PET drug prior to injection, but the maximum mass dose allowed will be 50 μg.

5. WARNINGS/PRECAUTIONS

[^{18}F]BF_4 is an experimental imaging agent that will be used at relatively low (tracer) doses. Because [^{18}F]BF_4 is in the early stages of clinical investigation, it is recommended that subjects receiving [^{18}F]BF_4 for injection be followed closely by means of adverse event reporting, vital signs and laboratory tests.

The potential for drug-drug interactions is not presently known. Until more data become available, it is recommended that drugs with a narrow therapeutic window not be co-administered with [^{18}F]BF_4.

There are no data on the effects of [^{18}F]BF_4 in human perinatal development. For this reason, fertile females must have a negative pregnancy test at screening to qualify for the study and a negative pregnancy test within 48 hours of administration of [^{18}F]BF_4 for injection. [^{18}F]BF_4 for injection must not be administered to females who are pregnant or lactating.

6. INVESTIGATIONAL PLAN

6.1 Overall Design and Plan of Study

This study will assess the biodistribution, as well as the pharmacokinetics of [^{18}F]BF_4 in 8 healthy volunteers (4 male, 4 female), 10 patients with myeloma, and 10 patients with endometrial cancer.

In healthy volunteers, screening assessments will take place over phone. Subjects who qualify for the study will be scheduled for a study visit within 30 days of screening. At the study visit, healthy subjects will have an initial physical exam, urine pregnancy test (if applicable) and will have catheters placed for i.v. drug administration and for blood sampling. Vital signs, and blood samples will be obtained. Subjects will then receive an infusion of 9-11 mCi [^{18}F]BF_4 for injection over 1 minute and PET imaging will begin. Three PET/CT scanning procedures will be performed over a period of approximately 4 hours with two rest breaks in between. Venous blood samples for clinical laboratory tests will be taken before radiotracer administration at 1.5 hours post-administration of radiotracer. In addition, three venous blood samples will be taken during the PET/CT scanning procedure to determine blood pharmacokinetics and metabolite evaluation. Urine collection will be performed immediately after each PET/CT scan.

For cancer patients undergoing virus treatments, subjects will undergo [^{18}F]BF_4-PET/CT imaging at baseline before virus administration and at day 9 following virus treatment. Subjects will be screened by Drs. Dispenzieri or Bakkum-Gamez within their clinics. Subjects who qualify for the study will return to the clinic within 30 days of screening, have a urine pregnancy test (if applicable) and will have catheters placed for i.v. drug administration. Vital signs will be obtained. All subjects will then receive a single i.v. bolus of 9-11 mCi [^{18}F]BF_4 for injection and PET/CT imaging will begin. Biopsies

will be performed to confirm NIS expression in tissue of tumor regions showing uptake of [^{18}F]BF_4 in up to five myeloma patients and five endometrial cancer patients when the site is accessible for biopsy.

6.2. Aim 2: Investigations with [^{18}F]BF_4 in Healthy Subjects

Healthy volunteers will be studied before cancer patients in Aim 3.

6.2.1. Imaging protocol

Subjects will be imaged at the following time points to collect biodistribution data:

Time Point	Image
-5 → 0	CT-Transmission Scan over heart
0 → 45	Dynamic PET Emission Scan over heart
45 → 110	1st Rest Period
110→ 120	Total Body CT-Transmission Scan
120 → 150	Total Body PET Emission Scan (including head)
150 → 200	2nd Rest Period
200 → 210	Total Body CT - Transmission Scan
210 → 240	Total Body Emission Scan (including head)

6.2.2 Planned Dosage and Duration of Treatment

6.2.2.1 Dosage and Administration

During this study, subjects will receive a single i.v. administration of 9-11 mCi sodium [^{18}F]BF_4 immediately prior to the first of three PET/CT imaging scans.

6.2.2.2 Rationale for Dosages

The proposed dose for this study is based on comparison to other known imaging agents and is supported by the dosimetry estimates generated from the biodistribution experiments following administration of [^{18}F]BF_4 to rodents. Preliminary dosimetry estimates suggest that the expected human exposure for most organs will be approximately 0.05 rem/mCi. The dose limiting organ is estimated to be the stomach with dose of 0.3 rem/mCi. The estimated human radiation exposure after a 11 mCi dose of [^{18}F]BF_4 (10.2 mSv) will be comparable or below the range for other approved imaging agents, such as ^{18}F-FDG.

[^{18}F]BF_4 will be formulated in 0.9% (w/v) sodium chloride solution and administered by i.v. bolus, in tracer amount of 9-11 mCi. The expected specific activity of [^{18}F]BF_4 is 1-10 GBq/μmol. We have set the acceptance criterion for specific activity as greater than 1 GBq/μmol. At a 9-11 mCi (333-407 MBq) human dose, the mass of [^{18}F]BF_4 will be 0.33-0.41 μmol or 36-44 μg. Higher doses may be administered to correct for the radioactive decay of PET drug prior to injection, but the maximum mass dose allowed will be 50 μg.

6.2.3 Selection of Healthy Subjects

6.2.3.1 *Inclusion Criteria*

1. Four male and four female healthy volunteers, greater than 21 years of age.
2. Subjects must provide written informed consent.
3. Willingness to provide all biological specimens as required by the protocol.

6.2.3.2 *Exclusion Criteria*

Volunteers with any of the following are ineligible to enroll in this study:

1. Have currently clinically significant cancer, neurologic, hepatic, renal, pulmonary, metabolic, or endocrine disturbances, especially thyroid disease;
2. Current clinically significant cardiovascular disease. Clinically significant cardiovascular disease usually includes one or more of the following:
   a. cardiac surgery or myocardial infarction within the last 6 months;
   b. unstable angina;
   c. coronary artery disease that required a change in medication within the last 3 months;
   d. decompensated congestive heart failure;
   e. significant cardiac arrhythmia or conduction disturbance, particularly those resulting in atrial or ventricular fibrillation, or causing syncope, near syncope, or other alterations in mental status;
   f. severe mitral or aortic valvular disease;
   g. uncontrolled high blood pressure;
   h. congenital heart disease;

3. History of drug or alcohol abuse within the last year, or prior prolonged history of abuse;
4. Clinically significant infectious disease, including AIDS or HIV infection or previous positive test for hepatitis B, hepatitis C, HIV-1, or HIV-2;
5. Women of childbearing potential must not be pregnant (negative urine β-HCG at the time of screen) or lactating over the course of the study. A commercial urine dipstick test will be performed within 48 hours prior to injection of [^{18}F]BF_4 unless the screening urine pregnancy test falls within 48 hours of injection.
6. Volunteers who, in the opinion of the investigator, are otherwise unsuitable for a study of this type;
7. History of severe drug allergy or hypersensitivity; or
8. Volunteers who had received an investigational medication within the last 30 days or who have participated in a clinical trial with any experimental medication or radiopharmaceutical in the last 30 days. Additionally, the time between the last dose of the previous experimental medication and enrollment (completion of screening assessments) must be at least equal to 5 times the terminal half-life of the previous experimental medication.
9. Volunteers who are taking drugs with narrow therapeutic windows, such as theophylline, or warfarin, heparin and other anticoagulant therapies

6.2.4 Prior and Concomitant Therapy

Except as noted below, all medications (prescription or over-the-counter) that have been started prior to screening may be continued during the course of the study. Attempts should be made to keep the dosage and administration stable throughout the study (from screening through the end of the imaging protocol).

All medications that are continued from the start of the study or that are started during the study (other than the study medication) must be documented in the case record form on the Concomitant Medication Page of the Case Record Form (CRF).

- Investigators must not enroll patients who are taking drugs with narrow therapeutic windows, such as theophylline, or warfarin, heparin and other anticoagulant therapies, and should not prescribe these drugs for the duration of the study.

6.2.5 Removal of Subjects from Study

Subjects must be removed from the study if:

1. Informed consent is withdrawn; or
2. The investigator believes it is in the best interest of the subject to be removed from the study.

Subjects may be withdrawn from the study if a serious adverse event occurs. The date and reason for discontinuation should be noted on the CRF. Subjects who discontinue prematurely should be seen for a final evaluation.

6.2.6 PROCEDURES AND METHODS FOR HEALTHY SUBJECTS

6.2.6.1. Screening: Day -30 - 0

Healthy subjects will be recruited from local population by local advertisements. Prospective subjects will contact the study coordinator by telephone (see attached recruitment phone script). If subject are deemed eligible for participation, signed informed consent will occur when they arrive for the study visit.

Screening assessments will be performed during this recruitment phone call and/or during a subsequent phone call within 30 days of the imaging session. Screening assessments, performed over phone, will include:

- Demographics (age, sex, education, alcohol and drug use, smoking);
- Medical history, concomitant medications;
- Disease history.

6.2.6.2 Subject Preparation

- Subjects will be asked to drink at least 2-8 oz glasses of water the morning of the test and prior to injection
- Subjects are not required to fast.

6.2.6.3 Observations and Imaging

The following assessments will be performed (see schedule of assessments 12.2 in Appendix):

- Informed consent
- Physical Exam. A physical examination will be conducted by a Nuclear Medicine physician at the subjects' arrival to the clinic and at the completion of the imaging study. Clinically significant changes that occur after injection of [^{18}F]BF_4 will be recorded as adverse events through the 24 hours post injection period.

- A pregnancy test (urine beta hCG, qualitative): will be performed at screening for females who are of childbearing potential. A commercial urine dipstick pregnancy test will be performed within 48 hours of injection if the serum pregnancy test falls outside the 48 hour window.

- Venous catheters will be established in both arms by a Nuclear Medicine Technologist prior to [^{18}F]BF_4 for injection.

- Subjects will be positioned in supine orientation in the GE 690XT PET/CT scanner. For attenuation correction and localization, CT of the same region will incorporate Helical CT with pitch of 1 and x-ray tube set to 120 kV and 70 mAs and CT of the body will incorporate Helical CT with a pitch of 1 and x-ray tube set to 120 kVp and 45 mAs.

- Immediately following administration of [^{18}F]BF_4 for injection (9-11 mCi over 1 min infusion period), subjects will undergo dynamic PET/CT imaging for 45 minutes over the heart with sequence 20x3s, 12x 10s, 4x30s, 5x60s, 7x300s. Following the dynamic PET/CT scan, the subjects will be asked to void the bladder and allowed to rest outside of the scanner for ~55 minutes (1st rest period). Trunk imaging by PET/CT will resume for 30 minutes (~2 h post-injection), followed by a second rest period for ~55 minutes. Trunk PET/CT imaging will resume for 30 minutes such that PET/CT scanning will be commenced at 210 min and completed at 240 min post-injection. Subjects will void urine during the rest periods and the entire void of urine will be collected in a pre-weighed jug for total radioactivity counts. Subjects will void at the end of study and a urine sample will be collected for total radioactivity counts.

If it becomes apparent in analyses of the data that the longer post-injection imaging times are not contributing valuable data, they may be dropped from further data collections after agreement between the study investigators. The investigators will prepare and distribute a written plan for image acquisition procedure and parameters as well as case report forms (CRFs) prior to the start of the study.

- Vital signs including blood pressure, oral temperature, pulse and respiration rate will be taken by a Nuclear Medicine Technologist immediately prior to administration of [^{18}F]BF_4 for injection, after the first PET/CT scanning sequence (~45 min post-injection), and at the end of the study (after completion of PET/CT scanning, ~240 min post-injection);

- Blood samples (venous) for pharmacokinetic and metabolic evaluation will be taken by a Nuclear Medicine Technologist at 40, 145, and 235 min after [^{18}F]BF_4 administration. Samples will be transported to the Molecular Imaging Research lab, Stabile 3-28, for processing.

- Urine samples will be collected by a Nuclear Medicine Technologist for total radioactivity counts after each PET/CT scan at approximately 60, 160, and 240 min post-injection of radiotracer. The mass (g) of urine collected at each time point will be recorded. The samples will be transferred together with the blood samples to Dr. DeGrado's lab in Stabile building for further analysis. The radioactivity concentration in urine (1 mL each) will be measured (mCi/mL) by using a well counter. The total radioactivity excreted in urine will be determined as the product of radioactivity concentration and urine volume (assuming a density of 1 g/ mL) for the three collection intervals.

- Blood samples will be collected by a Nuclear Medicine Technologist for clinical labs prior to administration of [^{18}F]BF_4 and at approximately 90 min post-administration of [^{18}F]BF_4 and be processed routinely in Mayo Laboratory Medicine and reported in the subjects medical record. The 1-2 needle sticks for these samples will be performed by Venipuncture Services. Three to four needles sticks will be made in total, including the sticks required for radiotracer injection and blood samples for radioactivity analysis. If the blood laboratory tests provide clinically meaningful information, the subject's primary physician will be notified.

The laboratory tests will include:

- Hematology (5 mL EDTA): hemoglobin, hematocrit, RBC, WBC, MCH, MCHC, neutrophils, bands, lymphocytes, monocytes, eosinophils, basophils, platelets, morphology, MCV, and RBC morphology.

- Chemistry (6 mL blood): total bilirubin, alkaline phosphatase, ALT (SGPT), AST (SGOT), urea nitrogen, creatinine, glucose, uric acid, calcium, phosphorus, total protein, albumin, total cholesterol, triglycerides, sodium, potassium, bicarbonate, chloride, magnesium, globulin, GGT, tryptase.

The analysis of the blood samples for [^{18}F]BF_4 levels and radiolabeled metabolites will be performed by collecting 3 mL samples in green top tubes and taken at multiple time points (40, 145, and 235 minutes). Samples will be immediately placed on ice to stop metabolism and/or transport into red blood cells. One mL of each blood sample will be pipetted to a 1.5 mL Eppendorf spin tube, spun down, and 0.4 mL plasma transferred by pipette to a test tube for counting. For counting of whole blood, 0.4 mL of each whole blood sample will be pipetted to test tube for counting. The radioactivity concentration in blood and plasma (1 mL each) will be measured (mCi/mL) by using a well counter.

- Subjects will be observed continuously for signs of adverse events or serious adverse events throughout the imaging study.

6.2.6.4 Adverse Event Follow-Up at 24 h post-administration of [^{18}F]BF_4

A scripted phone call will be made by a study coordinator to each subject at approximately 24 hours post-injection of [^{18}F]BF_4 to check for any adverse events. Any adverse events will be reported to the PI immediately following the phone call.

6.3. Aim 3: Investigations with [^{18}F]BF_4 in Cancer Patients

6.3.1. Imaging protocol

Subjects will be imaged before virus administration (baseline = Day -0) and Day 9 after administration of virus at the following time point to collect biodistribution data:

Time Point	Image
0	Inject [^{18}F]BF_4 (9-11 mCi)
90 → 95	Body CT Transmission Scan
95 → 135	Trunk PET Emission Scan

Adverse events and serious adverse events will be monitored continuously throughout the imaging study and through the 24 hour follow-up phone call. A Nuclear Medicine co-investigator physician must evaluate the subject at patient's arrival to the clinic for the imaging study and prior to discharge. Subjects who experience an adverse event will not be discharged until the event has resolved or stabilized.

6.3.2 Planned Dosage and Duration of Treatment

6.3.2.1 Dosage and Administration

During this study, subjects will receive two i.v. administrations of 9-11 mCi sodium [^{18}F]BF_4 immediately prior to imaging.

6.3.2.2 Rationale for Dosages

The proposed dose for this study is based on comparison to other known imaging agents and is supported by the dosimetry estimates generated from the biodistribution experiments following administration of [^{18}F]BF_4 to rodents. Preliminary dosimetry estimates suggest that the expected human exposure for most organs will be approximately 0.05 rem/mCi. The dose limiting organ is estimated to be the stomach with dose of 0.3 rem/mCi. The estimated human radiation exposure after a 11 mCi dose of [^{18}F]BF_4 (10.2 mSv) will be comparable or below the range for other approved imaging agents, such as ^{18}F-FDG.

[^{18}F]BF_4 will be formulated in 0.9% (w/v) sodium chloride solution and administered by i.v. bolus, in tracer amount of 9-11 mCi. The expected specific activity of [^{18}F]BF_4 is 1-10 GBq/μmol. We have set the acceptance criterion for specific activity as greater than 1 GBq/μmol. At a 9-11 mCi (333-407 MBq) human dose, the mass of [^{18}F]BF_4 will be 0.33-0.41 μmol or 36-44 μg. Higher doses may be administered to correct for the radioactive decay of PET drug prior to injection, but the maximum mass dose allowed will be 50 μg.

6.3.3 Selection of Subjects

6.3.3.1 *Inclusion Criteria*

6.3.3.1.1 Aim 3a. Myeloma Patients

1. Patients who are currently participating in Mayo Clinic MV-NIS trial (IRBs: 06-005263) and agree to enroll in additional [^{18}F]BF_4-PET studies.
2. Subjects are greater than 21 years of age.
3. Subjects must provide written informed consent.
4. All other inclusion criteria are met for study 06-005263.
5. Agree to provide biopsy of tumor indicated on PET for research purposes, if accessible;

6.3.3.1.2 Aim 3b. Endometrial Cancer Patients

1. Patients who are currently participating in Mayo Clinic VSV-hINFβ-NIS trial (IRB 15-007000) and agree to enroll in additional [^{18}F]BF_4-PET studies.
2. Subjects are greater than 21 years of age.
3. Subjects must provide written informed consent.
4. All other inclusion criteria are met for study 15-007000.
5. Agree to provide biopsy of tumor indicated on PET for research purposes, if accessible;

6.3.3.2 *Exclusion Criteria*

6.3.3.2.1 Aim 3a. Myeloma Patients

Patients with any of the following are ineligible to enroll in this study:

1. All exclusion criteria apply from study 06-005263.
2. Current clinically significant cardiovascular disease. Clinically significant cardiovascular disease usually includes one or more of the following:
   a. cardiac surgery or myocardial infarction within the last 6 months;
   b. unstable angina;
   c. coronary artery disease that required a change in medication within the last 3 months;
   d. decompensated congestive heart failure;
   e. significant cardiac arrhythmia or conduction disturbance, particularly those resulting in atrial or ventricular fibrillation, or causing syncope, near syncope, or other alterations in mental status;
   f. severe mitral or aortic valvular disease;
   g. uncontrolled high blood pressure;
   h. congenital heart disease;

   Before enrolling a patient with any of the above conditions, the co- investigator must contact the principal investigator
3. History of drug or alcohol abuse within the last year, or prior prolonged history of abuse;
4. Clinically significant infectious disease, including AIDS or HIV infection or previous positive test for hepatitis B, hepatitis C, HIV-1, or HIV-2;
5. Women of childbearing potential must not be pregnant (negative urine β-HCG at the time of screen) or lactating over the course of the study. A commercial urine dipstick test will be performed within 48 hours prior to injection of [^{18}F]BF_4 unless the screening urine pregnancy

test falls within 48 hours of injection.

6. Patients who, in the opinion of the investigator, are otherwise unsuitable for a study of this type;
7. History of severe drug allergy or hypersensitivity;
8. Patients who had received an investigational medication within the last 30 days or who have participated in a clinical trial involving medications other than the related MV-NIS or VSV-NIS viruses in the last 30 days. Additionally, the time between the last dose of the previous experimental medication and enrollment (completion of screening assessments) must be at least equal to 5 times the terminal half-life of the previous experimental medication.
9. Patients with current clinically significant medical comorbidities, that might pose a potential safety risk, interfere with the absorption or metabolism of the study medication or limit interpretation of the study results. These include but are not limited to clinically significant hepatic, renal, pulmonary, metabolic or endocrine disease, cancer, HIV infection and AIDS.
10. Patients who are taking drugs with narrow therapeutic windows, such as theophylline, or warfarin, heparin and other anticoagulant therapies

6.3.3.2.2 Aim 3b. Endometrial cancer patients

Patients with any of the following are ineligible to enroll in this study:

1. All exclusion criteria apply from VSV-hINFβ-NIS clinical trial (IRB 15-007000).
2. Current clinically significant cardiovascular disease. Clinically significant cardiovascular disease usually includes one or more of the following:
   a. cardiac surgery or myocardial infarction within the last 6 months;
   b. unstable angina;
   c. coronary artery disease that required a change in medication within the last 3 months;
   d. decompensated congestive heart failure;
   e. significant cardiac arrhythmia or conduction disturbance, particularly those resulting in atrial or ventricular fibrillation, or causing syncope, near syncope, or other alterations in mental status;
   f. severe mitral or aortic valvular disease;
   g. uncontrolled high blood pressure;
   h. congenital heart disease;

   Before enrolling a patient with any of the above conditions, the co- investigator must contact the principal investigator
3. History of drug or alcohol abuse within the last year, or prior prolonged history of abuse;
4. Clinically significant infectious disease, including AIDS or HIV infection or previous positive test for hepatitis B, hepatitis C, HIV-1, or HIV-2;
5. Women of childbearing potential must not be pregnant (negative urine β-HCG at the time of screen) or lactating over the course of the study. A commercial urine dipstick test will be performed within 48 hours prior to injection of [^{18}F]BF_4 unless the screening urine pregnancy test falls within 48 hours of injection.
6. Patients who, in the opinion of the investigator, are otherwise unsuitable for a study of this type;
7. History of severe drug allergy or hypersensitivity;

8. Patients who had received an investigational medication within the last 30 days or who have participated in a clinical trial with any experimental medication in the last 30 days. Additionally, the time between the last dose of the previous experimental medication and enrollment (completion of screening assessments) must be at least equal to 5 times the terminal half-life of the previous experimental medication.
9. Patients with current clinically significant medical comorbidities, that might pose a potential safety risk, interfere with the absorption or metabolism of the study medication or limit interpretation of the study results. These include but are not limited to clinically significant hepatic, renal, pulmonary, metabolic or endocrine disease, cancer, HIV infection and AIDS.
10. Patients who are taking drugs with narrow therapeutic windows, such as theophylline, or warfarin, heparin and other anticoagulant therapies

6.3.4 Prior and Concomitant Therapy

Except as noted below, all medications (prescription or over-the-counter) that have been started prior to screening may be continued during the course of the study. Attempts should be made to keep the dosage and administration stable throughout the study (from screening through the end of the imaging protocol).

All medications that are continued from the start of the study or that are started during the study (other than the study medication) must be documented in the case record form on the Concomitant Medication Page of the Case Record Form (CRF).

6.3.5 Removal of Subjects from Study

Patients must be removed from the study if:

1. Informed consent is withdrawn; or
2. The investigator believes it is in the best interest of the subject to be removed from the study.

Patients may be withdrawn from the study if a serious adverse event occurs. The date and reason for discontinuation should be noted on the CRF. Subjects who discontinue prematurely should be seen for a final evaluation.

6.3.6 PROCEDURES AND METHODS

6.3.6.1. Screening: Day -30 - 0

Patients will be selected from subjects electing to participate in IRB 06-005263 at Mayo Clinic: "Phase I/II Trial of Systemic Administration of Edmonston Strain of Measles Virus, Genetically Engineered to Express NIS, with or without Cyclophosphamide, in Patients with Recurrent or Refractory Multiple Myeloma", PI: Angela Dispenzieri, MD; or "Phase I Trial of Systemic Administration of Vesicular Stomatitis Virus Genetically Engineered to Express NIS and human interferon, in Patients with Metastatic and/or Incurable Endometrial and Epithelial Ovarian Cancer", PI: Jamie Bakkum-Gamez, MD, IRB 15-007000. Prospective subjects will have been screened for medical and physical compliance to the inclusion and exclusion criteria. If subject are deemed eligible for participation, participants will be invited to participate and sign informed consent. All screening assessments must be performed within 30 days of the imaging session. Screening assessments will include:

- Informed consent;
- Demographics (age, sex, education, alcohol and drug use, smoking);
- Medical history, physical exam, concomitant medications;
- Disease history (date/months since symptom onset, date/months since diagnosis);
- Vital signs

6.3.6.2 Subject Preparation – Baseline and Day +9 after virus administration

- Subjects will be asked to drink at least 2-8 oz glasses of water the morning of the test and prior to injection
- Subjects are not required to fast.

6.3.6.3 Observations and Imaging – Baseline and Day +9 after virus administration

The following assessments will be performed (see schedule of assessments 12.3 in Appendix):

- Physical Exam. A physical examination will be conducted by a physician at the patients' arrival to the clinic. Clinically significant changes that occur after injection of [^{18}F]BF_4 will be recorded as adverse events through the 24 hours post injection period.

- Subjects will void bladder

- Venous catheter will be established in one arm prior to [^{18}F]BF_4 for injection.

- Within a dimly lit uptake room, [^{18}F]BF_4 (9-11 mCi) will be administered as an infusion over 1 minute. Subjects will remain seated for a 90-min uptake period.

- Subjects will be position in supine orientation in the PET/CT scanner. For attenuation correction and localization, CT of the same region will incorporate Helical CT with pitch of 1 and x-ray tube set to 120 kV and 70 mAs and CT of the body will incorporate Helical CT with a pitch of 1 and x-ray tube set to 120 kVp and 45 mAs.

- Subjects will undergo a static trunk-region PET/CT imaging for 40 minutes.

- Biospy for histochemical confirmation of viral infection. The projected numbers of subjects that will undergo biopsy are shown in the table below:

Group	**n**
Myeloma w/o biopsy	5
Myeloma w/ biopsy	5
Endometrial Cancer w/o biopsy	5
Endometrial Cancer w/ biopsy	5

If there is an area that shows focal uptake in tumor on one of the [^{18}F]BF_4-PET scans, suggesting that there may be measles virus at that location, and if it is in a position in the body that is easy to biopsy, we will exercise the option, with allowance by the subject (see consent form) to perform a needle

biopsy to obtain tissue for pathological confirmation of viral infection. Dr. John Schmitz will perform the needle biopsy under guidance of ultrasound or CT, whichever is deemed more appropriate for the target location. Biopsies will be performed using standard sterile technique, local anesthesia, and potentially moderate sedation with fentanyl and midazolam when appropriate. As with all procedures, appropriate medication substitutions will be made based upon documented allergies or prior adverse reactions. Two 1-2 cm 18-gauge core samples will be obtained from the targeted tissue using a spring-loaded biopsy device. A 17-gauge introducer needle may be used to obtain access for the biopsy device. Immediate post-biopsy imaging will be obtained with the modality employed, as is standard for image guided procedures. The patient will be observed in the radiology post-procedure area according to established practice for site biopsied. The primary risks standard to all biopsies will be discussed with the patient including but not limited to: bleeding, non-target needle placement/biopsy, and adverse medication reaction. Biopsy sample will be analyzed for NIS expression by immunohistochemistry in the lab or Dr. Peng.

- Vital signs including blood pressure, oral temperature, pulse and respiration rate will be taken immediately prior to administration of [^{18}F]BF_4 for injection, and at the end of the study (after completion of PET scanning, ~135 min post-injection); Nuclear Medicine physician will perform a physical examination of patients before their release.

- Subjects will be observed continuously for signs of adverse events or serious adverse events throughout the imaging study.

6.3.6.4 Adverse Event Follow-Up – 24 h post-administration of [^{18}F]BF_4

A scripted phone call will be made to each subject at approximately 24 hours post-injection of [^{18}F]BF_4 to check for any adverse events.

7. Informed Consent and Human Subject Information

NOTE: Since all myeloma and endometrial cancer patients will be recruited from an existing pool of subjects already participating in studies at the Mayo Clinic, some of the screening information will already be accessible. A review preparatory to research application will used to review this data in advance of consent to determine eligibility.

All informed consent forms must be approved by Mayo Clinic Institutional Review Board (IRB). No study related procedures shall be performed prior to completion of the informed consent process, and signing of the consent form. A copy of the signed informed consent should be given to the patient and/or their caregiver for their records.

Subject will be exposed to radiation but the amount of radiation has a low risk of harmful effect. The amount of Sodium BF_4 used in the dose preparation is anticipated to be < 10 µg and no pharmacologic effect is anticipated. Women of childbearing potential must have a negative pregnancy test as part of their screening eligibility and within 48 hours of injection of the investigational drug [^{18}F]BF_4. Healthy subject will have 3-4 venous needle sticks to insert cannulas for drug injection and blood sample draws. Cancer patients will have one venous needle stick to insert cannula for drug injection. Subjects may experience pain, bruising or rarely infection at the

needle site. A total of 20 mL of blood will be drawn from healthy subjects. Urine samples will be collected in healthy subjects three times. Imaging may be tiring but every effort will be made to make subjects comfortable throughout the scan process. A head holder will be utilized to help subjects remain motionless.

Healthy subjects will be remunerated $200.00 and given parking passes for their time and inconvenience associated with study participation. Patients will be remunerated $300.00 and given parking passes for their time and inconvenience associated with the study protocol. Participants that do not complete the study will be provided partial remuneration according to the fraction of the study completed.

8. Documentation

PET scan results will be saved in electronic format (DICOM). All other data required by the protocol will be recorded in the CRF.

9. Adverse Events

9.1. Toxicity

Potassium [^{18}F]BF_4 was first reported by Anbar and Guttmann in 1960 (4). The original synthesis was performed by isotopic exchange, producing radiotracer with low specific activity (unreported value). Nevertheless, the radiotracer accumulated avidly into rat thyroid, consistent with the hypothesis of transport via NIS. Up to 1 mg of [^{18}F]KBF_4 was administered to rats with no noted toxicity. For $NaBF_4$, the acute subcutaneous LD50 in the rat was found to be 550 mg/kg (5, 6). The first human exposure to [^{18}F]BF_4 in the form of its potassium salt was reported by Entzian et al. in 1964 (7). Again, this radiotracer was synthesized in low specific activity (0.13 mCi/mg) by isotopic exchange. [^{18}F]KBF_4 (1-3 mCi, 7-23 mg) was administered to ten patients with brain tumors to determine potential to image brain tumors using an early coincidence rectilinear scanner. No adverse events were reported. A typical blood clearance curve was reported, showing a rapid clearance phase (T½ = 15 min) in the first 30 min, followed by a slower clearance phase out to 5 h (T½ = 290 min) (7). [^{18}F]KBF_4 was found to be primarily excreted in the urine with a excretion half-time of approximately 470 min. Further evidence of low toxicity of the BF_4^- anion is that it is contained in the formulations of a number of FDA approved drugs, including ^{99m}Tc-labeled Sestamibi (tetrafluoroborate is its counteranion), a myocardial perfusion imaging agent, which is administered in mg quantities to more than 6 million patients per year in the U.S. These data support the proposed studies with high-specific activity sodium [^{18}F]BF_4 (>50 mCi / mg = 10 GBq/μmol), where less than 10 μg of NaBF4 will be administered to each patient.

Based on this information, we do not anticipate any pharmacologic effect and therefore, no toxicities are anticipated in this study.

9.2. Adverse Event Monitoring

Each subject must be carefully monitored for adverse events. Healthy volunteers will be studied

before cancer patients. Blood samples will be analyzed in healthy subjects by a broad panel of clinical laboratory tests. Should any significant pharmacology or toxicity be identified in the healthy subjects in response to the test radiotracer, we will also include blood testing in cancer patients. An assessment will be made by a physician regarding the seriousness, intensity, and relationship to the administration of the study medication.

9.3. Adverse Event Definitions

Adverse Events

An adverse event is any undesirable experience occurring to a subject during the Phase 1/2 study, whether or not considered related to the investigational product.

For reporting purposes, study staff will distinguish among pre-existing conditions, study-emergent adverse events, and treatment-emergent adverse events.

Pre-existing conditions (i.e., undesirable experiences, signs, or symptoms that begin prior to the Screening Visit) will be recorded on the medical history and/or physical exam portion of the Case Report Form. These conditions will not be entered on the adverse event portion of the Case Report Form unless they worsen in intensity or frequency after the Screening Visit.

Study-emergent adverse events are undesirable experiences, signs or symptoms that begin, or worsen in intensity or frequency, after the Screening Visit, and prior to administration of study drug at the imaging visit. These will be recorded on the adverse event pages.

Treatment-emergent adverse events are undesirable experiences, signs, or symptoms that begin or worsen in intensity or frequency after the administration of study drug.

Serious Adverse Event

A serious adverse event is an adverse event that is fatal or life-threatening, or results in hospitalization, prolongation of hospitalization, persistent or significant disability/incapacity, or a congenital anomaly/birth defect. A life-threatening adverse event is an adverse event that, in the view of the investigator, places the subject at immediate risk of death from the reaction, as it occurred. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse events when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Relationship to Investigational Product

The assessment of the relationship of an adverse event to the administration of the study drug (remote, possible, and probable) is a clinical decision based on all available information at the time of the completion of the CRF. The following definitions of the relationship between the study drug and the adverse event (including serious adverse events) should be considered:

- *Remote (unlikely, doubtful, improbable):*
  The time course between the administration of the study medication and the occurrence or worsening of the adverse event makes a causal relationship unlikely and another cause (concomitant drugs, therapies, complications etc.) is probable.
- *Possible:*
  The time course between the administration of the study medication and the occurrence or worsening of the adverse event is consistent with a causal relationship but another cause (concomitant drugs, therapies, complications etc.) cannot be ruled out;

  <or>

  The time course between the administration of the study medication and the occurrence or worsening of the adverse event is not consistent with a causal relationship but no alternative cause can be identified.

- *Probable:*

  The time course between the administration of the study medication and the occurrence or worsening of the adverse event is consistent with a causal relationship and no other cause can be identified (concomitant drugs, therapies, complications etc.)

If the Investigator is unable to assess causality, the adverse event should be considered "Possible" by definition and not "Remote."

Intensity/Severity of an Adverse Event

In addition to assessing the relationship of the administration of the investigational product to adverse events, an assessment is required of the intensity (severity) of the event.

The following classifications should be used:

- *Mild:*
  A mild adverse event is an adverse event, usually transient in nature and generally not interfering with normal activities.
- *Moderate*:
  A moderate adverse event is an adverse event that is sufficiently discomforting to interfere with normal activities.

- *Severe:*
  A severe adverse event is an adverse event that incapacitates the subject and prevents normal activities. Note that a severe event is not necessarily a serious event. Nor must a serious event necessarily be severe.

9.4. Adverse Event Documentation

All adverse events must be fully recorded on the Adverse Event Page of the Case Report Form. Laboratory test abnormalities considered by the Investigator to be clinically relevant should be reported on the Adverse Event page of the Case Report Form. Signs and symptoms of each AE

should be described in detail (e.g., start and stop dates, severity, intensity, relationship to investigational product, action taken, and outcome).

Adverse events and laboratory test abnormalities fulfilling the definition of a serious adverse event should, in addition, be reported on the Serious Adverse Event Reporting Form.

Mayo Clinic lab standards and clinically determined normal values will be used by the study investigators to determine how and if results meet adverse event standards.

9.5. Reporting of Serious Adverse Events

Serious adverse events must be reported to Mayo Clinic IRB within 5 days of learning of it, using the IRB Reportable Event form. All reportable events meeting the Unanticipated Problem Involving Risk to Subjects or Others (UPIRTSO) criteria as submitted to the IRB using the Reportable Event form will be sent to an IRB Chairperson for review and determination. If the IRB Chairperson determines the event is a UPIRTSO, a convened IRB reviews it and either confirms or does not confirm the determination. The investigator is notified and the review, determination, and investigator communication is documented in IRB electronic system. A UPIRTSO, as confirmed by the convened IRB, is reported to the Mayo Clinic Institutional Official and other relevant Federal agencies, when required, within 30 days from the date of submission. The investigator is electronically notified of this action.

9.6 Reporting of AEs and SAEs to the FDA

All adverse reactions probably attributable to the use of the radioactive drug in the research study shall be immediately (within 7 days) reported by the Radioactive Drug Research Committee to the Food and Drug Administration, Center for Drug Evaluation and Research, HFD-160, 5600 Fishers Lane, Rockville, MD 20857.

10. STATISTICAL ANALYSIS

10.1. Objectives

The primary objective of this study is to evaluate the uptake and distribution, and metabolism of the imaging agent [^{18}F]BF_4. This is a preliminary study and all analyses will be exploratory in nature.

10.2. Data Sets

All subjects that received [^{18}F]BF_4 for injection will be included in the analysis. Tissue uptake, biodistribution and metabolite analysis will include all subjects for whom there is sufficient data to evaluate the parameter in question. Where possible, tumor uptake will be quantified by use of the Standardized Uptake Value = (tissue concentration) / (injected dose/body wt. (g)).

10.3. General Descriptive Statistics

For each of the three cohorts of participants recruited, general descriptive statistics will be calculated. The descriptive statistics will include mean, standard deviation, median, and ranges for continuous variables, and frequency and percent frequency for categorical variables. In addition, graphical displays of the data (e.g., box plots, density plots, etc) will be prepared to better understand the scaling of data within each cohort.

10.4. Statistical Analysis Plan

10.4.1 Primary Analysis

Healthy Volunteers:
An exploratory ANOVA model with blocking factor for patient will be used to assess effect of gender on [^{18}F]BF_4 biodistribution. Biological half-times in major tissues will be estimated from the 3 imaging time points.

Cancer Patients:
Biodistribution of uptake in SUV will be descriptively summarized within each patient cohort. An exploratory ANOVA model with blocking factor for patient and within patient measurements corresponding to measurement site will be fit to the data. Contrasts will be used to compare uptake in regions where there should be increased uptake (e.g., tumor site) and should not (normal tissue).

In addition to the summarization of the general uptake, uptake in known or suspected lesions will be conducted in the two disease cohorts. Specifically, both [^{18}F]BF_4-PET and ^{99m}Tc-SPECT scans (acquired from separately approved IRB protocols, see note on page 5) will be analyzed by a Nuclear Medicine Physician/Radiologist for the following:

1. Number of lesions visualized (areas of positive uptake within tumor relative to background).
2. Size range of lesions visualized.
3. Lesion contrast with surrounding structures using the 4-point scale below:

0: No uptake in tumor observed
1: Low uptake in tumor observed equivalent to background
2: Moderate uptake in tumor observed higher than background
3: High uptake in tumor observed relative to background

4. Reader confidence using the 4-point scale below:
0: unsure
1: somewhat confident
2: moderately confident
3: very confident

5. For tumors that show uptake with PET, the amount of uptake will be quantified. PET allows quantitative estimation of SUV = (tissue concentration)/(injected dose/body wt. (g)). SPECT does not allow absolute quantification.

As with the general uptake analysis described above, descriptive statistics will be used to quantity these attributes. For regions of interest (ROIs) with corresponding biopsy data available, a preliminary diagnostic accuracy study will be conducted. Given the limited patient data available, ROIs will be assumed to be statistically independent (i.e., we will conduct a lesion-level analysis without accounting for clustering in patients). There is the potential for verification bias if the PET/SPECT data is used to guide the biopsy process. This is a limitation of the study design. Receiver operating characteristic (ROC) curves will be generated using the lesion contrast scale.

10.4.2 Safety Analysis

Adverse Events

Any adverse event which occurs within 24 hours of administration of [^{18}F]BF_4 will be reported. The investigator shall immediately report to the Radioactive Drug Research Committee all adverse effects associated with the use of the radioactive drug in the research study.

Adverse events will be assessed on a patient-by-patient basis as well as aggregation over all participants studied. For the latter, AEs will be summarized in terms of number and percentage of patients experiencing an adverse event. The summary will be further broken down by body system and preferred term using Common Toxicity Criteria (CTC) terms. Adverse events will also be presented by severity, relationship to treatment, and seriousness. All patients that experience serious adverse events or that discontinue due to adverse events will be summarized.

Discontinuation

All subjects who discontinued participation prior to completing the study will be listed and their discontinuation reasons will be tabulated.

Laboratory Data

Clinically significant changes in laboratory data from Baseline will be summarized. Subjects whose laboratory values are outside threshold values will be identified and tabulated.

Vital Signs

Clinically significant changes in vital signs from baseline will be summarized.

10.5. Image Analysis

Healthy Volunteers:
Descriptive statistics will be used to evaluate changes in time of uptake of [^{18}F]BF_4 in the heart lung and blood pool over time from time of injection to 45 min in healthy subjects. Biodistribution in other organs will be evaluated from the subsequent trunk PET/CT images out to 4 h post-administration. Tissue uptake will be normalized to injected dose through conversion of image data to Standardized Uptake Value [SUV = (tissue radioactivity concentration/cc tissue) / (injected dose / g body mass)]. The regions used for SUV determination will be of a standardized volume for a representative region within each tissue type, and the SUV averaged over that region. Thus, all subjects will have similar sized regions for each tissue type, minimizing the effect of region size.

Cancer Patients:
In myeloma and endometrial cancer patients, tumor, muscle, and other tissue SUVs will be assessed at baseline and at Day +9. Primary assessments will be made as described above (10.4.1). Changes in this SUV assessment from baseline to Day +9 will be analyzed by ANOVA. We will also do similar statistical analysis on tumor:muscle ratios. Radiation dosimetry estimates will be determined from the data in healthy human subjects using OLINDA software, with both male and female groups.

10.6. Metabolite data

Healthy Volunteers:
The amount of ^{18}F-radioactivity in the form of metabolites other than [^{18}F]BF_4 will be measured by TLC in plasma over the 4 hour imaging period. Appropriate metabolic half-live will be calculated and descriptive statistics presented.

10.7 Sample Size Justification

This study is not a "powered" study with statistically tested hypotheses. Descriptive statistics will be used for measuring biostribution in the healthy subjects. As such, statistical 'rules of thumb' are used to justify the sample size. Using the 'rule of thumb' that recommends using at least 10 observations per parameter in a regression model, a sample size of 10 myeloma patients and 10 endometrial cancer patients is proposed in order to estimate ANOVA statistics. The sample size for health controls is reduced to 8 with anticipating of pooling the data across patient cohorts and minimizing unnecessary exposure.

11. INVESTIGATOR'S REGULATORY OBLIGATIONS

11.1. Investigational Product Control

The receipt of clinical supplies (i.e. starting material for [^{18}F]BF_4) must be documented at the site.

All drug supplies for this study should be retained in a safe and secure place at all times during the study. [^{18}F]BF_4 for injection should be prepared by a qualified radiopharmacist/chemist under GMP conditions and dispensed by the pharmacist, the investigator, or by a qualified individual under the investigator's supervision. An up-to-date dispensing record must be maintained.

The PI will cooperate with Mayo Clinic Radiation Safety office for all necessary documentation needed for conducting the studies under the auspices of the Mayo Clinic Radioactive Drug and Research Committee (RDRC). The Mayo Clinic Radiation Safety office manages all communications with the FDA concerning documentation required for RDRC studies.

12. APPENDICES

12.1. References

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12.2 STUDY FLOW CHART

	Day 1											Day 2
	Minutes Post [^{18}F]BF_4 Administration											
Time Points	-30	-5	Dose	0-45	45-110	110-120	120-150	150-200	200-210	210-240	240+	
EVALUATIONS												
Signed Consent	X											
Medical/AD History	X											
Concomitant Meds	X											
Vital Signs[1]	X				X 45 min						X	
Height	X											
Weight	X											
Clinical Labs[2]	X				X 90 min							
Nuc Med Eval	X										X	
Temperature	X										X	
Urine Pregnancy Test[3]	X											
[^{18}F]BF_4 Injection			X									
Venous blood for Radioactivity				X 40 min			X 145 min			X 235 min		
Urine for Metabolites					X 60 min			X 160 min			X 240 min	
PET Imaging		Heart CT Trans	Heart Emission dynamic scan 0-45 minutes		~55 REST PERIOD			~55 min REST PERIOD				
Biodistribution						Body Trans	Body Emission		Body Trans	Body Emission		
Adverse Event Monitoring			X----------									X
24 hour AE Phone Call												X

1. Includes blood pressure, oral temperature, pulse and respiration rate
2. Includes Hematology (hemoglobin, hematocrit, RBC, WBC, MCH, MCHC, neutrophils, bands, lymphocytes, monocytes, eosinophils, basophils, platelets, morphology, MCV and RBC morphology) and Chemistry (total bilirubin, alkaline phosphatase, ALT(SGPT) AST (SGOT), blood urea nitrogen, creatinine, glucose, uric acid, calcium, phosphorus, total protein, albumin, total cholesterol, triglycerides, sodium, potassium, bicarbonate, chloride, magnesium, globulin, GGt and tryptase) testing.
3. Not required if screening urine pregnancy test results are within 48 hours prior to [^{18}F]BF_4 injection

This protocol is the same for baseline (Day -0) and Day +9 post-virus administration

12.3 STUDY FLOW CHART

	Baseline (Day -0) and Day +9 post-virus administration								Following day
	Time (min)								
Time Points	Screen	-30	-5	0 Dose	0-90	90-95	95-135	135	
EVALUATIONS									
Signed Consent	X								
Medical/AD History	X								
Concomitant Meds	X								
Vital Signs[1]	X	X						X	
Height	X								
Weight	X	X							
Nuc Med Eval		X						X	
Temperature		X							
Urine Pregnancy Test	X	X[2]							
[^{18}F]BF_4 Injection				X					
PET/CT Imaging				Uptake period		CT Imaging	PET Imaging		
Adverse Event Monitoring				X----------					----------X
24 hour AE Phone Call									X

1. Includes blood pressure, oral temperature, pulse and respiration rate
2. Not required if screening urine pregnancy test results are within 48 hours prior to [^{18}F]BF_4 injection