Evaluating *in vivo* PARP-1 expression with ^{18}F-FluorThanatrace positron emission tomography (PET/CT) in Breast Cancer

Principal Investigator(s): Elizabeth McDonald, MD, PhD
Assistant Professor of Radiology
Department of Radiology
University of Pennsylvania

Sub-Investigators: Robert Doot, PhD, Radiology
Michael Farwell, MD, Radiology
Daniel Pryma, MD, Radiology
Austin Pantel, MD, Radiology
Kara Maxwell, MD, PhD, Oncology
Robert Mach, PhD, Radiology
David Mankoff, MD, PhD, Radiology
Amy Clark, MD, Oncology
Payal Shah, MD, Oncology
Angela DeMichele, MD, Oncology
Julia Tchou, MD, Surgery
Michael Feldman, MD, PhD, Pathology
Sean Carlin, PhD, Radiology
Anupma Nayak, MD, Pathology
Theresa Berger, MBE, Radiology
Erin Schubert, BS, Radiology
Mehran Makvandi, PhD, Radiology
Maha Ayub, Radiology
Matthew Fillare, Radiology
Jarrod Goodarz, Radiology

IND Sponsor The Department of Radiology, University of Pennsylvania, School of Medicine

Table of Contents

Study Summary

Title	**Evaluating *in vivo* PARP-1 expression with ^{18}F-FluorThanatrace positron emission tomography (PET/CT) in Breast Cancer**
Short Title	[18F]FTT in Breast Cancer
Phase	Phase I pilot study
Study Design	Patients with primary breast or metastatic breast cancer with at least one lesion that is ≥ 1.0 cm in size may be eligible for this study. Only one type of standard imaging needs to show a lesion at least 1 cm in size in order for a subject to be eligible for study participation (e.g. mammography, ultrasound, CT, FDG PET/CT or MRI). Patients may participate in this study if they are at least 18 years of age. We anticipate enrolling up to 30 participants with breast cancer who meet eligibility requirements for this study. Positron emission tomography (PET/CT) imaging will be used to evaluate PARP-1 activity in sites of breast cancer using the investigational radiotracer [18F]FTT. This is an observational study in that [18F]FTT PET/CT will not be used to direct treatment decisions. While patients and referring physicians will not be blinded to the [18F]FTT PET/CT results, treatment decisions are made by the treating physicians based upon clinical criteria. 30 patients who will undergo approximately 60 minutes of dynamic scanning followed by skull base to mid-thigh scan imaging post injection of [18F]FTT starting at approximately 60 minutes post injection. PET/CT imaging sessions will include an injection of approximately 10 mCi (approximate range for most studies is anticipated to be 8-12 mCi) of [18F]FTT. Data will be collected to evaluate uptake of [18F]FTT in breast cancer and compare with PARP-1 activity in tissue, when available.
Study Center(s)	University of Pennsylvania
Objectives	**Primary Objectives** • Evaluate PARP-1 activity in breast cancer using measures of uptake of [18F]FluorThanatrace **Secondary Objectives** • Correlate [18F]FluorThanatrace uptake measures with PARP-1 IHC activity in the biopsy specimen. • Correlate [18F]FluorThanatrace uptake measures with hormone receptor status • Evaluate the safety of [18F]FluorThanatrace • Correlate [18F]FluorThanatrace uptake measures with PARP-1 [125I]KX1 autoradiography activity in the surgical specimen. • Correlate [18F]FluorThanatrace uptake measures with germline and tumor mutation status
Number of Subjects	Up to 30 evaluable participants will enroll in this imaging study at the University of Pennsylvania for one imaging cohort

Diagnosis and Main Inclusion Criteria	**Inclusion Criteria** 1. Participants will be ≥ 18 years of age 2. Primary breast or metastatic breast cancer 3. At least one lesion that is 1 cm or greater in size by standard imaging (e.g. mammography, ultrasound, CT, FDG PET/CT or MRI). Only one type of imaging is required to show a lesion of 1 cm or greater in order for the patient to be eligible to participate in this study. Patients that have a prior diagnosis of primary breast cancer in the opposite breast can be included. 4. Willing to allow use or collection of tissue/blood for the purposes of research from either clinical biopsy or surgical procedure (if adequate tissue is available) or research only biopsy/blood draw 5. Participants must be informed of the investigational nature of this study and be willing to provide written informed consent and participate in this study in accordance with institutional and federal guidelines prior to study-specific procedures. **Exclusion Criteria** 1. Females who are pregnant or breast feeding at the time of screening will not be eligible for this study; a urine pregnancy test will be performed in women of child-bearing potential at screening. 2. Inability to tolerate imaging procedures in the opinion of an investigator or treating physician *3.* Any current medical condition, illness, or disorder as assessed by medical record review and/or self-reported that is considered by a physician investigator to be a condition that could compromise participant safety or successful participation in the study
Study Product	[18F]FluorThanatrace ([18F]FTT)
Statistical Methodology	Data for the primary analysis will be summarized using descriptive statistics (number of subjects [N], mean, standard deviation [SD], median, quartiles, minimum, and maximum) for continuous variables and using frequency count and percentage for discrete variables. The demographic and baseline characteristics data will be summarized for all subjects. Safety data will be summarized for all subjects. PARP activity as assayed from biopsy specimens will be correlated with uptake within tumor on imaging.

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

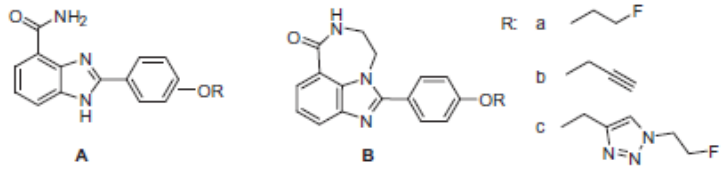
1. Background

1.1. Investigational Agent

1-(4-(2-Fluoroethoxy)phenyl)-8,9-dihiydro-2,7,9a-triazabenzo[*cd*]azulen-6(7*H*)-one also known as [18F]FluorThanatrace or [18F]FTT is a positron emitting

radiopharmaceutical that has been studied in animals for selective measurement of the in vivo inhibition of the PARP-1 nuclear enzyme with positron emission tomography (PET/CT). Zhou et al described synthesis of a series of radiolabeled benzimidazole carboxamide analogs that could be easily labelled with ^{18}F and their inhibition potency against PARP-1 was determined [1]. Newly synthesized PARP-1 inhibitors were assessed for their ability to inhibit active PARP-1 using the method described by Putt and Hergenrother[2](Table 1). The results showed that tricycle benzamide analogs had higher inhibition potency than their respective benzimidazole analogs. The analogs with fluoroethoxy substituent had three times higher inhibition potencies than the respective analogs having a fluoroethyl triazole group. From this, the most potent inhibitor, **12**, was selected for ^{18}F-labeling.

Table 1
Inhibition efficiency of PARP-1 inhibitors



Compound	Structure	R	IC_{50} (nM)
1 PJ34	/	/	170.2 ± 8.3[a]
8	A	a	10.8 ± 0.4
9	A	b	25.8 ± 3.3
10	A	c	30.3 ± 5.6
12	B	a	6.3 ± 1.3
13	B	b	18.7 ± 2.7
15	B	c	22.1 ± 6.3

[a] Reported values: IC_{50} = 20 nM, EC_{50} = 35 nm.[3,24]

Fluorine-18 labeled FluorThanatrace [^{18}F]FTT can be synthesized with high specific activity so the quantity of mass injected with the radiopharmaceutical is < 10 µg. This proposed protocol will evaluate the feasibility of using [^{18}F]FTT as an *in vivo* measure of PARP-1 in tumors.

1.2. Rationale

POLY(ADP-RIBOSE) POLYMERASE (PARP) INHIBITORS

Poly(ADP-ribose) polymerase (PARP) is a family of enzymes involved in base excision repair (the repair of DNA single-strand breaks) and alternative end joining (repair of DNA double-strand breaks). The molecular basis of PARP1 inhibitor function may depend on the dual roles of PARP1 as a modulator of gene transcription in addition to DNA damage repair [3]. Inhibition of PARP results in persistent single-strand DNA breaks which are subsequently converted to double strand breaks. Double strand breaks can be repaired via homologous recombination, non-homologous end joining and alternative end joining. In BRCA-mutated tumors and other tumors with defects in DNA repair, PARP inhibition can lead to genetic errors and instability with subsequent cell death since homologous recombination is not active and non-homologous end joining is error prone [4].

Pre-clinical Studies of PARP Inhibitors

Combining PARP synergistically with radiation for cellular killing has been investigated since the 1980's [5,6]. The recent development of more specific and potent PARP inhibitors have spurred human trials based on provocative preliminary data: In 2005, Alan Ashworth and others published the observation that inhibition of PARP activity in BRCA1 and BRCA2 mutated cells resulted in chromosomal instability, cell cycle arrest and apoptosis [7-9]. They demonstrated that inhibition of PARP created persistent single strand breaks in BRCA deficient cells, normally repaired by homologous recombination. Without a viable mechanism of repair, BRCA cells would selectively undergo cellular lethality. Subsequent *in vivo* studies demonstrated that PARP inhibition selectively blocked the growth of BRCA-2 deficient tumors [7,8]. Thus, it was hypothesized that targeting the DNA repair defect in BRCA mutant human tumors might be a selective therapeutic strategy.

Early Clinical Trials

Based on strong pre-clinical data, selective PARP inhibitors were developed and tested on multiple cancer types including BRCA1 and 2 mutated ovarian and breast cancer. Eventually, first in man studies of the PARP1/2 inhibitor, olaparib, as a single agent in advanced cancers were conducted. One early Phase 1 trial evaluating olaparib enrolled any patient with refractory cancer but anti-tumor activity was only observed in patients with known BRCA mutations [10]. Subsequent expansion cohorts focused on patients with BRCA mutations with continued demonstration of progression free benefit only in these select patients [11,12]. Continued incremental benefit in patients with BRCA mutation was demonstrated in a randomized phase II study of olaparib in patients with recurrent serous ovarian cancer [13,14].

Preclinical studies:

The highly potent PARP-1 inhibitor, **1-(4-(2-Fluoroethoxy)phenyl)-8,9-dihiydro-2,7,9a-triazabenzo[*cd*]azulen-6(7*H*)-one also known as [18F]FluorThanatrace or [18F]FTT** was developed based on the tricycle benzimidazole core by Zhou et al [1]. **12** was radiolabelled with [18F] to create a positron emission tomography (PET) imaging agent. **[18F]FTT** is a radiolabeled PARP-1 inhibitor that is capable of imaging PARP-1 activity *in vivo* with positron emission tomography (PET/CT). [18F]FTT toxicology studies in animals have been completed and indicate that at the tracer microdose amounts proposed for this study, [18F]FTT is unlikely to have any pharmacological effect or expected toxicity in humans. We hypothesize that [18F]FTT is a highly specific radiotracer that may be useful for evaluating *in vivo* PARP-1 activity in various cancers. Published data suggest that the *in vivo* properties of [18F]FTT are optimal for measuring PARP-1 activity in tumors and this radiotracer should be further developed for human studies.

1.3. Risk/Benefit assessment

[18F]FluorThanatrace ([18F]FTT) is an investigational imaging drug, the radiosynthesis and *in vivo* evaluation of the radiotracer has been previously reported by Zhou et al, as a radiolabeled PARP-1 inhibitor for measuring PARP-1 expression *in vivo* with PET demonstrating promising results in animal models. Human dosimetry for this novel radiotracer has also been calculated.

The [18F]FTT dose proposed for this study will be approximately 10 mCi (approximate range at injection for most studies is anticipated to be 8 - 12 mCi) the mass of FluorThanatrace to be injected will be ≤ 10 µg. Based on preclinical toxicity studies performed in Sprague-Dawley rats where FluorThanatrace was well tolerated by rats and the no observable adverse effect level (NOAEL) following a single IV bolus was 0.863 mg/kg. Using the body surface area conversion factor of 6.2 for rats, the Human Equivalent Dose (HED) was calculated to be 0.139 mg/kg. The proposed dose for human studies meets the definitions provided by the FDA for a "microdose" as less than 1/100th of the dose of test substance calculated from animal data to yield pharmacologic effect of the test substance, with a maximum allowed dose of ≤ 100 µg for radiopharmaceuticals and it will be studied under an FDA exploratory IND (128,178-IND). There have been no AEs in the current human PARP trial at Penn with 15 patients scanned successfully with administered doses ranging from 8.59-11.65 mCi, and we will use the same FTT dose range as in the ongoing trial (8-12 mCi).

In the circumstance that a participant has an AE, the principal investigator will determine the severity of the AE and the relationship of the event to radiotracer administration and decide the course of action and appropriate treatment or follow up for the study subject.

Risk/Benefit

[18F]FTT is a positron emitting radiopharmaceutical. As such, it poses an intrinsic radiation exposure risk. However, when administered in low "microdose" tracer amounts as a PET imaging agent, as described in this protocol, this risk is felt to be small.

Initial human dosimetry studies have been conducted at the University of Pennsylvania in 4 adult females with ovarian cancer (ages 46-70, mean 59) that included PET/CT scans evaluated for 4 hours post intravenous administration of [18F]FTT (mean 401 MBq, 366-431 MBq (10.8 mCi, 9.9-11.6 mCi)). The highest activity was seen in the spleen with an average radiation dose of 0.436 ± 0.183 mSv/MBq. The average effective dose was 0.023 ± 0.002 mSv/MBq.

Human radiation doses calculated from the PET images, indicated a mean effective dose of 8.5 mSv (0.023 ± 0.002 mSv/MBq) for the protocol's target injected dose of 370 MBq (10 mCi) of [18F]FTT which is commensurate with standard [18F]FDG PET imaging and other clinical nuclear medicine procedures that are widely accepted. Based on this data calculations were made an injected dose of up to 12 mCi will yield acceptable organ and total body doses associated with [18F]FTT PET/CT imaging that are felt to be comparable to those associated with other published PET/CT biodistribution data for a variety of [18F]-labeled compounds. Four human subjects were studied and initial data analysis of the biodistribution for these subjects was found to show acceptable human dosimetry estimates for this novel radiotracer that was consistent with pre-clinical data.

This research involves exposure to radiation that could affect a fetus or pregnant woman, or breast-feeding baby due to this risk urine or serum pregnancy test will be performed for women of childbearing potential at the time of screening. Women who are pregnant or breast-feeding at the time of screening will not be included in this study. A urine pregnancy test will be repeated within one day of the PET/CT scan for all women of childbearing potential, if a pregnancy test is found to be positive the subject will be discontinued from the study.

There is potential with intravenous injections, including [18F]FTT, for allergic reactions. The dose will be delivered intravenously by skilled clinical professionals and subjects will be monitored for any signs or symptoms of allergic reaction by trained personnel during the PET procedure. Symptoms of an allergic reaction could include hives, shortness of breath or difficulty breathing.

Venous cannulation is a routine clinical procedure that carries minimal risks when performed by trained personnel. It is possible that bruising, dizziness or fainting could occur in some subjects. There is a risk of phlebitis or infection, which is very remote.

The PET/CT scan takes place in a small, enclosed space and therefore can be uncomfortable for some people with claustrophobia or musculoskeletal disorders (such as arthritis). Subjects will be made as comfortable as possible and PET technologists and study personnel will available throughout the imaging to address any discomfort. The subject will be allowed to get off the table between the described imaging segments as necessary.

Incidental Findings: It is possible that during the course of the research study, the research staff may notice an unexpected finding(s). Should this occur, the finding(s) will be considered by the appropriate personnel and the PI will determine if the patient should be informed. These possible finding(s) may or may not be significant and may lead to anxiety about a condition and to further work-up by the subject's physician.

If subjects consent for an optional research biopsy the biopsy procedures will be carried out by trained personnel at the University of Pennsylvania. The type of biopsy performed will be an imaging guided core needle biopsy. The optimal biopsy method will be determined by a physician. Risks of biopsy include discomfort or bruising at the biopsy site, bleeding from the biopsy site, pain, redness or swelling at the biopsy site and infection at the biopsy site, which is rare.

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

There is no anticipated benefit to study subjects as a result of their participation in this study. There is potential benefit to general society if [18F]FTT PET/CT proves to be a useful imaging agent for evaluation of PARP-1 activity in tumors.

2. Study Objectives

2.1. Primary Objectives

- Evaluate PARP-1 activity in breast cancer using measures of uptake of [18F]FluorThanatrace

2.2. Secondary Objectives

- Correlate [18F]FluorThanatrace uptake measures with PARP-1 IHC activity in the biopsy specimen
- Correlate [18F]FluorThanatrace uptake measures with hormone receptor status
- Evaluate the safety of [18F]FluorThanatrace

- Correlate [^{18}F]FluorThanatrace uptake measures with PARP-1 [^{125}I]KX1 autoradiography activity in the surgical specimen.
- Correlate [^{18}F]FluorThanatrace uptake measures with germline and tumor mutation status

3. Study Design

This is a pilot study in patients with primary breast or metastatic breast cancer with at least one lesion that is ≥ 1 cm by standard imaging (e.g. mammography, ultrasound, CT, FDG PET/CT or MRI) only one type of imaging needs to show a lesion size ≥ 1 cm in order for the patient to be eligible to participate in this study. Patients may participate in this study if they are at least 18 years of age, most participants will be receiving care at the clinical practices of the University of Pennsylvania. Patients who come to the University of Pennsylvania for diagnosis and/or treatment of breast cancer and who meet the study inclusion criteria may be approached by study personnel for recruitment into this study. Patients will be approached about study participation regardless of race or ethnic background. We anticipate enrolling up to 30 participants with breast cancer who meet eligibility requirements for this study.

After undergoing screening assessments and verifying eligibility for one study participation in one of the subject cohorts, subjects will undergo an experimental [^{18}F]FTT PET/CT scan. If a patient is going to undergo surgery after their first scan, tumor tissue may be obtained for research purposes if there is enough tissue available for research.

Study imaging will be performed using a whole-body PET/CT scanner (Philips Medical Systems, Netherland). The protocol will be performed under the regulatory approval of the (Institutional Review Board) IRB and FDA x-IND. Each patient will receive ≤ 12 mCi of [^{18}F]FTT intravenously (approximate range for most studies is anticipated to be 8-12 mCi). Patients will undergo dynamic PET/CT imaging over the chest (including the breasts). The dynamic scan will take approximately 60 minutes and will be followed by a skull base to mid-thigh scan beginning at approximately 60 minutes post-injection. The purpose of the static scan is to image the extent of active disease the number of fields of view imaged may vary depending on the patient's size. The static torso scan will be optional at the discretion of an investigator or study physician. If a patient is unable to tolerate the additional time on the scanner or for any reason the investigator deems it necessary to omit it, if the static torso scan is omitted this will be documented in the CRF and will not be considered a protocol deviation.

All patients will be included in analyses of the primary objectives but may not be included in analyses of all the secondary objectives. Most primary breast patients typically have their cancer surgery scheduled within 4 weeks after they were first seen in clinic as part of their standard care, however, no specific timing for the surgery is required by this protocol. The research PET/CT scan will be completed after enrollment to the study in the time available prior to a breast surgical procedure or the initiation of therapy.

Patients will be asked permission to access their medical records for follow up of their clinical care and treatment as part of the consent for this study. This access includes results of the standard breast cancer pathology immunohistochemistry (IHC) panel (usually estrogen receptor (ER), progesterone receptor (PgR) and HER2/neu), which will be collected from the medical record for both primary breast cancer biopsy and surgical resection when available. For subjects that undergo sentinel node biopsy or axillary dissection as part of a primary breast surgery we will record pathology results available

from the lymph nodes. There are no planned additional research pathology tests that will be done on lymph node tissue. Tests ordered for routine clinical care will be given priority if limited tissue is available and all proposed assays will likely not be performed on all patients. Prioritization of research assays in cases where limited tissue is available for research purposes will be determined by an investigator with the aid of pathology colleagues.

4. Participant Selection

4.1. Eligibility Criteria

4.1.1. Inclusion Criteria

1. Participants will be ≥ 18 years of age
2. Primary breast or metastatic breast cancer
3. At least one lesion that is 1 cm or greater in size by standard imaging (e.g. mammography, ultrasound, CT, FDG PET/CT or MRI). Only one type of imaging is required to show a lesion of 1 cm or greater in order for the patient to be eligible to participate in this study.
4. Willing to allow use or collection of tissue/blood for the purposes of research from either clinical biopsy or surgical procedure (if adequate tissue is available) or research only biopsy/blood draw
5. Participants must be informed of the investigational nature of this study and provide written informed consent in accordance with institutional and federal guidelines prior to study-specific procedures.

4.1.2. Exclusion Criteria

1. Females who are pregnant or breast feeding at the time of screening will not be eligible for this study; a urine pregnancy test will be performed in women of child-bearing potential at screening.
2. Inability to tolerate imaging procedures in the opinion of an investigator or treating physician
3. Serious or unstable medical or psychological conditions that, in the opinion of the investigator, would compromise the subject's safety or successful participation in the study.

Only individuals (aged 18 or over) who can understand and give informed consent will be approached to participate in this study. Individuals who are considered to be mentally disabled will not be recruited for this study. All subjects must understand and be able to give informed consent. We will not be using specific methods to assess decisional capacity. All individuals will be told that their choice regarding study participation will in no way change their access to clinical care. This should negate any undue influence or coercion. Children, fetuses, neonates, or prisoners are not included in this research study. The menopausal status of the study participant will be reviewed during the screening process by examining the patient's chart, women of child-bearing potential will have a serum or urine pregnancy test at the time of screening.

4.2. Subject Recruitment and Screening

Patients who come to the clinical practices of the University of Pennsylvania for diagnosis and/or treatment of breast cancer and who meet the study inclusion criteria may be approached by study staff for recruitment into this study. Eligibility criteria will be checked and study personnel will go over the consent form and the patient will be given an opportunity to have any questions answered by study staff or a study investigator some of this initial process may occur over the phone prior to in person written consent. After providing written informed consent the participant will undergo screening assessments, these may be done on more than one day. The screening assessments are described in detail in Section 6 “Study Procedures and Study Calendar”.

All patients being considered for the study and who are eligible for screening will sign an informed consent for the study prior to any study specific procedures. Following completion of the screening assessments and confirmation of eligibility, patients will undergo the [18F]FTT PET/CT scan before they undergo surgical resection of a primary breast cancer or start of systemic therapy. Patients may have other clinical standard imaging done as part of their standard care (e.g. MRI, ultrasound, mammography, CT, bone scan, FDG PET/CT) that may be reviewed as part of determination of eligibility and for the purposes of image comparison with the [18F]FTT PET/CT scans.

A two-part consent process will be used for this study and the consent for administration of the investigational drugs will be discussed again with the patient on the day of the PET/CT scan and documented on a supplemental consent form by a delegated Nuclear Medicine Authorized User prior to administration of any study drugs.

4.3. Subject Withdrawal

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

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

Follow –up information will be obtained for subjects who discontinue the study if possible.

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

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

5. Investigational Agent

5.1. Description

1-(4-(2-Fluoroethoxy)phenyl)-8,9-dihiydro-2,7,9a-triazabenzo[*cd*]azulen-6(7*H*)-one also known as [18F]5, [18F]FluorThanatrace or [18F]FTT is a positron emitting radiopharmaceutical that has been studied in animals for selective measurement of the in vivo inhibition of the PARP-1 nuclear enzyme with positron emission tomography (PET/CT). Zhou et al described synthesis of a series of radiolabeled benzimidazole carboxamide analogs that could be easily labelled with ^{18}F and their inhibition potency against PARP-1 was determined [1]. Newly synthesized PARP-1 inhibitors were assessed for their ability to inhibit active PARP-1 using the method described by Putt and Hergenrother [2](Table 1). The results showed that tricycle benzamide analogs had higher inhibition potency than their respective benzimidazole analogs. The analogs with fluoroethoxy substituent had three times higher inhibition potencies than the respective analogs having a fluoroethyl triazole group. From this, the most potent inhibitor, **12**, was selected for ^{18}F-labeling.

Fluorine-18 labeled FluorThanatrace [18F]FTT can be synthesized with high specific activity so the quantity of mass injected with the radiopharmaceutical is < 10 µg.

5.2. Preparation of Study Drug

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

5.3. Receipt of Study Drug [18F]FluorThanatrace ([18F]FTT)

[18F]FTT will be delivered to the Nuclear Medicine Division of the University of Pennsylvania Medical Center by a trained Cyclotron team member in single dose vials according to the standard procedures outlined by the Cyclotron Facility. Once the drug has been delivered to the Nuclear Medicine Division all standard hospital procedures will apply for handling, processing, and destruction of any residual amounts if applicable. As [18F]FTT is a short-lived radiotracer with a half-life of approximately 110 minutes it will be

synthesized for same day use, [18F]FTT will not be stored. The remaining activity in the vials that exists in the lab will be stored only until the activity decays to undetectable level. All vials will be disposed of according to the standard operating procedure set forth by the hospital.

5.4. Study Drug Administration:[18F]FluorThanatrace ([18F]FTT)

The [18F]FTT dose will be drawn and activity measured in the dose calibrator in the imaging facility and administered by bolus injection to the patient under the direct supervision of a Nuclear Medicine Authorized User. The injectable dose of [18F]FTT for most studies will be approximately 10 mCi (approximate range for most studies is anticipated to be 8-12 mCi), a lesser dose may be injected if, in the opinion of a Nuclear Medicine Authorized User complete imaging data could be generated. In the dose of [18F]FTT, only a small fraction of the FTT molecules are radioactive. [18F]FTT is administered to subjects by intravenous injection of ≤ 16.5 mL. The injection and/or imaging procedure will be terminated in any patient who exhibits anaphylaxis, significant dyspnea or chest pain.

6. Study Procedures and Study Calendar

STUDY CALENDAR

	Screening	Baseline Assessments
Informed Consent	X	
Demographics	X	
Pregnancy Test (if applicable)[1]	X	X
[18F]FTT PET/CT		X[2]
Collection of research tumor tissue		X
Long term follow up[3]		

[1]For women of childbearing potential a urine pregnancy test will be performed within 1 day prior to injection of [18F]FTT

[2] [18F]FTT PET/CT will take place prior to surgical resection of a primary breast cancer or before starting systemic therapy. Adverse event follow up after the PET/CT scan may occur by telephone or in person, depending on the subject's schedule. Follow up should take place the next available business day (i.e. not a weekend or a holiday). The AE monitoring period is 24 hours following [18F]FTT injection

[3]Long term follow up will occur by medical record review for the duration of the subject's cancer care until death or 15 years after enrollment.

6.1. Intake session and screening assessments

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

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

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

Patients may have a standard imaging ordered as part of their clinical care (e.g. CT, MRI, ultrasound, FDG PET/CT) ordered by a treating physician as part of standard medical care, these scans will likely occur before the patient is consented and enrolled in this study. Correlative pertinent imaging may be reviewed by an investigator if necessary if the referring physician is unsure from available clinical reports whether there is at least one site of disease that is ≥ 1.0 cm.

Many patients will undergo biopsies as part of their clinical care. Standard pathology results from clinical biopsy will be obtained from medical record review. We may also ask permission to access tissue collected as part of clinical biopsy or surgical procedure, if available. If the patient gives permission, archived tissue may be accessed for the purposes of this study to perform additional experimental pathology assays in addition to genomic testing. For patients with primary surgical resection, if there is adequate tissue available after the clinical diagnostic needs are met, we will collect two small samples of tissue with the aid of the pathology staff who can identify appropriate tissue sections. In addition, subjects will be enrolled in The Breast Program- Translational Cancer Resource (TRACR) biobank protocol and agreed to collection of biosamples and/or data as part of that protocol. Permission to access biosamples from the biopsy, blood draws and/or surgical resection which have been collected as part of the TRACR protocol for purposes including genetic testing will be part of the consent for this study. In order to identify available specimens and link to collected clinical data our study records will include the master TRACR ID. The tissue specimens and associated data will be tracked through the caTISSUE Core and ACCARD database maintained by research personnel as part of the TRACR study. Of note, small breast cancers (especially those that require needle localization) are generally not suitable for tissue banking as the entire specimen must be submitted for pathology evaluation. Therefore, not every patient that is eligible to participate in this study will have studies done on fresh tissue. If there is not adequate tissue available for collection at the time of the clinical procedure then no research tissue will be collected and stored. If this occurs, this will be noted in the CRF. This will not be considered a protocol deviation and the patient's imaging data will still be analyzed for primary endpoint analyses. However, the subject's data will be censored from correlations of imaging data with pathology markers of PARP activity or somatic mutation analysis. Prior to analysis, samples will be stored at -80C in a dedicated research freezer.

Translational studies will then be performed on the tumor tissue including PARP activity levels and somatic sequencing. Germline sequencing will also be performed, if possible, to determine mutational status. To measure PARP activity, fresh frozen tumor tissue may be analyzed using the Universal Colorimetric PARP assay kit from Trevigen which measures the incorporation of biotinylated poly(ADP-ribose) onto histone proteins and related assays [15]. Appropriate positive and negative controls will be utilized. This will be performed through the laboratory of Dr Robert Mach. Digital autoradiography for PARP1 enzyme expression in tumor samples may be performed using fresh, frozen tissue.

Sections will be prepared and digital autoradiography will be performed on a cryotome machine in Dr. Robert Mach's lab located in the Chemistry building, similar to our ongoing ISO-1 protocol (*IRB # 820737, UPCC # 18114)*. Any unused samples will be stored in the lab of Dr Robert Mach or Dr. Sean Carlin.

The following additional patient data may be self-reported by the patient or obtained from medical chart review: demographics (including gender, date of birth and race), menopausal status, BRCA mutation status, if known. A urine pregnancy test will be done at the time of screening, before FTT injection in women of child-bearing potential.

6.2. Scheduling Phone Call

A member of the study staff or investigator will provide potential subjects with a copy of the consent form. If a potential subject is not able to meet in person prior to the day of the planned study imaging then study staff or an investigator will call the potential subject to review the study, they will explain the study procedures, provide detailed information about potential risks and benefits of participating in the study and confirm the patient's understanding of the research protocol. Any questions the subject has will be answered fully. If the patient confirms they are interested in participating in the study verbal consent for the scheduling of the imaging study and ordering the investigational product will be obtained by the study personnel during the phone call. This verbal consent for scheduling will be documented. The study personnel or investigator will emphasize that participation in the study is voluntary and that the study participant may choose to withdraw from this study at any time without jeopardizing current or future treatment. Written informed consent will be obtained in person prior to any study specific procedures being performed.

6.3. Day of [^{18}F]FluorThanatrace ([^{18}F]FTT PET/CT

Consent for administration of the investigational drugs will be discussed again with the patient on the day of the PET/CT scan and documented on a supplemental consent form by a delegated Nuclear Medicine Authorized User prior to administration of any study drugs.

The following procedures will be done on the day of [^{18}F]FTT PET/CT scans:

Weight and height will be recorded. A urine pregnancy test will be performed within 1 day prior to the injection of [^{18}F]FTT in all women of child-bearing potential.

PET/CT imaging procedure

Prior to positioning on the PET/CT scanner the patient will be allowed to urinate if necessary. The patient will be positioned on the PET/CT scanner. A brief low-dose CT scan will be acquired according to standard PET/CT imaging procedures. This scan is used for attenuation correction and anatomical localization of findings in the PET scan. There are no separate diagnostic CT scans performed as part of this research.

One intravenous (IV) line will be placed prior to start of scanning, this will generally be placed in the arm opposite the side of the primary breast cancer. A dose of approximately 10 mCi (approximate range for most studies is anticipated to be 8-12 mCi) of [^{18}F]FTT will be administered by IV injection to the patient under the direct supervision

of a Nuclear Medicine Authorized User. A lesser activity may be injected if, in the opinion of a nuclear medicine authorized user, complete imaging data could be generated.

Patients will undergo dynamic PET/CT imaging for approximately 60 minutes over the chest (including the breasts). This will be followed by a skull base to mid-thigh scan beginning at approximately 60 minutes post-injection. The purpose of the static scan is to image the extent of active disease, the number of fields of view imaged may vary depending on the patient's size. The static torso scan will be optional at the discretion of an investigator or study physician and may be omitted if a patient is unable to tolerate the additional time on the scanner or for any reason the investigator deems it necessary to omit it. If the static torso scan is omitted this will be documented in the CRF and will not be considered a protocol deviation. All images will be reconstructed using standard reconstruction techniques.

6.4. Follow-up Phone Call Session

Adverse events will be recorded for the period up to 24 hours post injection of the radiotracer. Research personnel will conduct follow up by telephone or in person, depending on the subject's schedule. Follow up should take place the next available business day (i.e. not a weekend or a holiday).

7. Image Interpretation

7.1. [18F]FTT PET/CT Image Analysis

The overall quality of the generated images will be evaluated. Initial imaging of the skull base to liver will be used to investigate regional tracer uptake in tumor and normal tissues for this novel radio tracer. Uptake and retention of [18F]FTT in known sites of tumor will be evaluated visually by trained radiology readers, the principal investigator or designee, a number of analysis methods may be tested to identify the optimal method for analyses for future studies with this radiotracer.

The experimental [18F]FTT results are observational and will not be used to make treatment decisions.

[18F]FTT PET/CT scans will be interpreted both qualitatively and quantitatively.

Qualitative interpretation will be recorded as positive or negative as defined below.

- Qualitative Positive – All known lesions show [18F]FTT uptake visible above background
- Qualitative Negative – No known lesions show [18F]FTT uptake visible above background

Quantitative interpretation will be recorded as max, mean and peak Standard Uptake Value (SUV) for the primary breast lesion (if primary is still in place, more than one lesion may be recorded if there is multi-focal disease).

8. Statistical Plan

8.1. Sample Size Determination

Sample size was based on the pilot nature of this exploratory study. A total of up to 30 evaluable patients will be accrued to the study. Prior studies suggest that an n = 30 is an adequate number of subjects to characterize a new radiotracer [16] [17] [18] [19] [20]. The main objectives of this study are demonstrate the feasibility of using the novel PARP-1 imaging agent [18F]FTT to image breast tumors in humans. Subjects who are enrolled but are unable to complete the entirety of their FTT imaging procedure will be considered evaluable for the primary endpoint of the study (uptake versus IHC biopsy specimen), but possibly not for the secondary endpoints.

8.2. Statistical Methods

For this early exploratory pilot study of feasibility data will be collected and summarized using descriptive statistics (number of subjects [N], mean, standard deviation [SD], median, quartiles, minimum, and maximum) for continuous variables and weighted using frequency count and percentage for discrete variables. The demographic and baseline characteristics data will be summarized for all subjects. Safety data will be summarized for all subjects.

To test the association of [18F]FTT uptake with phenotypic breast cancer subtypes (e.g. HER2+, TN, ER/PR+, etc.), a chi square test will be performed; estimates of sensitivity and specificity for each subtype will be reported. To assess the correlation between [18F]FTT uptake and qualitative IHC staining the null hypothesis that there is no association between FTT uptake (SUVmax) and PARP-1 expression, versus the alternative hypothesis that there is an association will be tested. For 80% power and 5% type I error rate (two-tailed), 30 patients will be needed to detect a correlation of at least 0.49 between FTT uptake and PARP-1 expression.

We will collect information from the medical record regarding long term outcomes (progression free survival and overall survival) for fifteen years after study enrollment.

9. Adverse Events

9.1. Definitions

Adverse Events are classified as serious or non-serious.
Adverse Events that do not meet the established criteria for Serious Adverse Events (see below) should be regarded as ***non-serious adverse events***.

Adverse Event
An ***adverse event*** (AE) is any untoward medical occurrence in a participant that does not necessarily have a causal relationship with the study procedure. It is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study procedure. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

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

Serious Adverse Event
A ***Serious Adverse Event*** (SAE) is any Adverse Event that is:
- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Expected and Unexpected Adverse Events
AEs may be **expected** or **unexpected**:

- An **expected AE** is one that is described in the protocol, the ICF, or the investigator's brochure.
- An **unexpected AE** is one that has not been described in the protocol, the ICF, or the investigator's brochure.

Adverse Event Grading
Grade denotes the severity of the AE.

1 – Mild
2 – Moderate
3 – Severe
4 – Life-threatening or disabling
5 – Fatal

Adverse Event Attribution
Attribution is used to determine whether an AE is related to a study treatment or procedure.
Attribution categories are:

Definite: The AE is **clearly related** to a treatment or procedure
Probable: The AE is **likely related** to a treatment or procedure
Possible: The AE **may be related** to a treatment or procedure
Unlikely: The AE is **likely unrelated** to a treatment or procedure
Unrelated: The AE is **clearly not related** to a treatment or procedure

Important Medical Events
Important Medical Events are those events that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject's health and may require medical intervention.

Preexisting Condition

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

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-Study Adverse Event
All unresolved adverse events should be followed by the investigator until the event is resolved or the subject is lost to follow-up.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if the Principal Investigator believes the abnormality is related to participation in the study.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be recorded as a serious adverse event. The event should be reported if the Principal Investigator believes the hospitalization is related to participation in the study.

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 24 hours following the administration of the study radiotracer,[^{18}F]FTT.

9.2. Recording of Adverse Events

At the PET/CT imaging session, the Principal Investigator or designee must seek information from the subject in reference to any adverse events specific to imaging procedures or injection of [^{18}F]FTT that may have occurred, by specific questioning and, as appropriate, by examination. Information on adverse events should be recorded, in as much detail as possible, in a source document or the medical record and entered on the Adverse Event Log.

The PI should be contacted immediately to determine the reporting requirements for the event as outlined in the protocol.

The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the investigational agent or study procedure is not the cause of the event.

9.3. Reporting of Adverse Events and Serious Adverse Events

It is the responsibility of the Principal Investigator to determine the grade, attribution and expectedness of the event and if the event is reportable according to the requirements outlined in the protocol.

For this investigational agent the following criteria should be met to consider an adverse event reportable:

- The event occurred within 24 hours after the injection of the investigational agent, [^{18}F]FTT.
- All unexpected events regardless of grade or attribution.
- Expected events that are grade 3 or higher.
- Expected events that are possibly, probably or defiantly related to the investigational agent.

When reporting please supply a narrative with as much description of the event as possible (chart note is acceptable) along with the completed AE Log or CRF signed and dated by the Principal Investigator.

The narrative should include:

- Protocol number and name
- Subject number
- Date of onset
- A detailed description of the event
- If the study procedure was discontinued

9.3.1. Investigator Reporting: Notifying the Study Sponsor

All reportable Adverse Events and Serious Adverse Events must be reported to the study sponsor (The Department of Radiology, IND Support Office) by email within 24 hours of the event. Please include the AE Log and narrative as mentioned above.

If the event is considered serious the Principle Investigator must complete the Serious Adverse Event (SAE) form and fax or email to the study sponsor within 24 hours. All documentation will be kept on file at the study site.

Significant new information on ongoing adverse events should be provided promptly to the study sponsor

9.3.2. Investigator Reporting: Notifying the Penn IRB

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

The Penn IRB requires Principal Investigators to submit AE and SAE reports within 10 working days from the time the investigator becomes aware of the event.

The Penn IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below:

- The event occurred within 24 hours after the injection of the investigational agent.

- All unexpected events regardless of grade or attribution.
- Expected events that are grade 3 or higher.
- Expected events that are possibly, probably or defiantly related to the investigational agent.

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

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

Other Reportable Events to Penn IRB:
For clinical drug trials, the following events are also reportable to the Penn IRB:

- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

9.3.3. Investigator reporting: notifying the Cancer Center DSMC

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

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

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

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

9.4. Sponsor Reporting

Notifying the FDA

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

- ***Within 7 calendar days***
  Any study event that is:
  - Associated with the use of the study drug.
  - Unexpected.
  - Fatal or life-threatening.

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

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

Notifying Participating Investigators

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

Additional Sponsor Reporting Requirements

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

9.5. Medical Monitoring

It is the responsibility of the Principal Investigator, Dr. McDonald, to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 11 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events and determination of whether there is any increase in risk to other subjects participating in the study related to the occurrence of an SAE.

10. Data Handling and Record Keeping

10.1. Confidentiality

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

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

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

All research personnel associated with this study have completed the University of Pennsylvania's Patient Oriented Research Training Program as well as HIPAA Compliance Training. Trained staff will assess eligibility, introduce the study rationale, procedures, study risks, and collect the combined informed consent/HIPAA authorization

form. The study team will work to uphold the privacy of the participants in several ways. Communications made among study staff regarding participants will use ID numbers whenever possible and minimize the use of patient name or other identifying information except when necessary for conduct of the study. Paper-based records will be kept in a secure location and only be accessible to personnel involved in the study. Computer-based files will only be made available to personnel involved in the study through the use of access privileges and passwords. Whenever feasible, identifiers will be removed from study-related information. In data analysis sets, we will use ID numbers and/or patient initials only.

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

1. Name
2. Address
3. Electronic mail addresses
4. Telephone Number
5. Date of Birth
6. Medical Record Number
7. Health Plan ID numbers
8. Social Security Number
9. Any other unique identifying number, characteristic, or code
10. Current and past medications or therapies
11. Information from a physical examination that generally includes blood pressure reading, heart rate, and temperature
12. Information from the tests and procedures described earlier in this document
13. Previous procedure, diagnosis and treatment information from institutions other than Penn if it relates to study eligibility
14. Emergency contact number, name, and relationship

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

10.2. Source Documents

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

10.3. Records Retention

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

of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

11. Study Monitoring, Auditing, and Inspecting

11.1. Study Monitoring Definition

Study Monitoring is the oversight of the progress of a research study and verifying that it is conducted, recorded, and reported in accordance with the protocol, standard operating procedures, Good Clinical Practice (GCP), and federal regulations. Monitoring oversees protocol and regulatory compliance, participant's welfare and safety. Monitoring is a recurring observation of a study as it progresses.

11.2. Study Monitoring

The Study Sponsor is responsible for assigning staff to monitor the research study. The Study Monitor will conduct all monitoring activities in accordance with the Study Monitoring Plan. Study Monitors will review all required regulatory documents, subject charts and Case Report Forms (CRFs) for completion and accuracy.

11.3. Study Monitoring Plan

The Study Monitoring Plan will be prepared prior to activation of the study and will be implemented after accrual to the study. The frequency and intensity of monitoring activities depend on several factors, including risk to study subjects, complexity of the study protocol, and implications of the research findings. The higher the risk to subjects, the more frequently monitoring will be conducted. Other factors that may increase monitoring frequency include the projected enrollment number, the anticipated rate of subject accrual and protocol compliance.

11.4. Study Monitoring Visits

The Principal Investigator will allocate adequate time and space for monitoring activities. The Principal Investigator will also ensure that the Study Monitor or other Compliance or Quality Assurance Reviewer is given access to all requested study-related documents and facilities if necessary.

Monitoring visits usually take place where the study is being conducted. There will be occasions where monitoring will take place in the IND Support office.

Monitoring Visits are usually composed of Study Initiation Visit, Interim Visits and the Study Close-out Visit. Principal Investigators will be informed of upcoming Visits and will be given adequate time to prepare for a Monitoring Visit.

Frequency of Visits

Enrollment will be complete when all 30 subjects are enrolled in the study. Monitoring visits will be conducted periodically throughout the study as described below:

- The **first monitoring visit** will occur after the first two subjects from each group have completed their research imaging visit.

- A **second monitoring visit** will be conducted when approximately 15 of the subjects from each group have completed their research imaging visit.

- A **third monitoring visit** will be conducted after 30 of the subjects have completed all of their research imaging visits. This visit may also serve as the close-out monitoring visit. This visit may also serve as the close-out monitoring visit.

Data Review

Subject charts and Case Report Forms (CRFs) for the first 2 subjects of each group will be reviewed at the first monitoring visit. Subject charts and CRFs on 4 randomly selected subjects will be reviewed at the second and third visits for each group.

11.5. Documentation of the Monitoring Visit

All monitoring visit findings will be documented on the Monitor's Report and a copy will be sent to the Principle Investigator. Any findings from the visit will require resolution within 30 working days

11.6. Auditing and Inspecting

The Principal Investigator will also permit study-related audits and inspections required by any University Compliance and/or Quality Assurance group, the EC/IRB and any government regulatory body including the FDA and according to their timeline.

12. Ethical Considerations

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

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

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

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study,

will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

13. References

1. Zhou D, Chu W, Xu J, et al. Synthesis, [(1)(8)F] radiolabeling, and evaluation of poly (ADP-ribose) polymerase-1 (PARP-1) inhibitors for in vivo imaging of PARP-1 using positron emission tomography. *Bioorganic & medicinal chemistry.* 2014;22(5):1700-1707.
2. Putt KS, Hergenrother PJ. An enzymatic assay for poly(ADP-ribose) polymerase-1 (PARP-1) via the chemical quantitation of NAD(+): application to the high-throughput screening of small molecules as potential inhibitors. *Analytical biochemistry.* 2004;326(1):78-86.
3. Feng FY, de Bono JS, Rubin MA, Knudsen KE. Chromatin to Clinic: The Molecular Rationale for PARP1 Inhibitor Function. *Mol Cell.* 2015;58(6):925-934.
4. Walsh CS. Two decades beyond BRCA1/2: Homologous recombination, hereditary cancer risk and a target for ovarian cancer therapy. *Gynecol Oncol.* 2015;137(2):343-350.
5. Durrant LG, Boyle JM. Potentiation of cell killing by inhibitors of poly(ADP-ribose) polymerase in four rodent cell lines exposed to N-methyl-N-nitrosourea or UV light. *Chem Biol Interact.* 1982;38(3):325-338.
6. Nduka N, Skidmore CJ, Shall S. The enhancement of cytotoxicity of N-methyl-N-nitrosourea and of gamma-radiation by inhibitors of poly(ADP-ribose) polymerase. *Eur J Biochem.* 1980;105(3):525-530.
7. Bryant HE, Schultz N, Thomas HD, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature.* 2005;434(7035):913-917.
8. Farmer H, McCabe N, Lord CJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature.* 2005;434(7035):917-921.
9. McCabe N, Turner NC, Lord CJ, et al. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. *Cancer research.* 2006;66(16):8109-8115.
10. Fong PC, Boss DS, Yap TA, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med.* 2009;361(2):123-134.
11. Audeh MW, Carmichael J, Penson RT, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. *Lancet.* 2010;376(9737):245-251.
12. Tutt A, Robson M, Garber JE, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. *Lancet.* 2010;376(9737):235-244.
13. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N Engl J Med.* 2012;366(15):1382-1392.
14. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol.* 2014;15(8):852-861.
15. Huang D, Yang C, Wang Y, Liao Y, Huang K. PARP-1 suppresses adiponectin expression through poly(ADP-ribosyl)ation of PPAR gamma in cardiac fibroblasts. *Cardiovascular research.* 2009;81(1):98-107.
16. Petrou M, Frey KA, Kilbourn MR, et al. In vivo imaging of human cholinergic nerve terminals with (-)-5-(18)F-fluoroethoxybenzovesamicol: biodistribution, dosimetry, and tracer kinetic analyses. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine.* 2014;55(3):396-404.
17. Mukherjee J, Christian BT, Dunigan KA, et al. Brain imaging of 18F-fallypride in normal volunteers: blood analysis, distribution, test-retest studies, and preliminary assessment of sensitivity to aging effects on dopamine D-2/D-3 receptors. *Synapse.* 2002;46(3):170-188.
18. Kimura H, Kawai T, Hamashima Y, et al. Synthesis and evaluation of (-)- and (+)-[(1)(1)C]galanthamine as PET tracers for cerebral acetylcholinesterase imaging. *Bioorganic & medicinal chemistry.* 2014;22(1):285-291.

19. Joshi BP, Wang TD. Imaging: Dynamic imaging of gut function--allowing the blind to see. *Nature reviews Gastroenterology & hepatology.* 2014;11(10):584-586.
20. Mankoff DA, Tewson TJ, Eary JF. Analysis of blood clearance and labeled metabolites for the estrogen receptor tracer [F-18]-16 alpha-fluoroestradiol (FES). *Nuclear medicine and biology.* 1997;24(4):341-348.