ASSESSMENT OF FUNCTIONAL STATUS OF ESTROGEN RECEPTORS IN BREAST CANCER BY POSITRON EMISSION TOMOGRAPHY

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Study Drug: PET imaging tracer ^{18}F-FFNP

Applicable IND(s): ^{18}F-FFNP IND # 76,214 Investigator Initiated Dr. Farrokh Dehdashti

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Protocol Revision History

Initial Approval Version **12/03/2014**

Description of Change	**Protocol Location**
20 August 2014 Summary of Changes after PRMC Initial Review	
Added description of who is providing estradiol	5.3 Estradiol Challenge Test
Added description of risks associated with the study amount of estradiol	5.4 Risks Related to Estradiol Challenge Test.
Clarified that the changes in SUV and T/N after treatment will be compared in responders and nonresponders between the baseline and post estradiol challenge FFNP-PET/CT images.	6.4 Image Analysis
Clarified that clinical response using RECIST version 1.1 by an oncologist will be used.	6.6 Evaluation of Tumor Response
Added blood draws to study calendar	STUDY CALENDAR
Updated Data Submission schedule to match source document labels	Data Submission Schedule
Updated protocol version date	Bottom of each page
08 December 2014 Summary of Changes IRB Initial Review	
Added exclusion criteria for subjects with spinal lesions at risk for cord compression in the opinion of the Principal Investigator and treating medical oncologist.	3.2 Exclusion Criteria
Added HRPO #, updated version date and protocol #	Title Page
28 April 2015 Amendment 1	
Added study title to schema and clarified in treatment with endocrine therapy should be planned for a minimum of 6 months	SCHEMA
Updated table of contents	Page 4
Clarified inclusion of pre-menopausal patients, clarified inclusion criterion to include locally advanced / metastatic patients, added endocrine therapy should be planned to be administered for at least 6 months, clarified prior therapy is allowed if ≥ 12 months since last treatment	3.1 Inclusion Criteria
Clarified both FFNP-PET/CT scans are completed prior to the start of standard of care endocrine therapy	5.2 Patient Population
Added expected toxicities to estradiol challenge	6.6 Toxicities Related to Estradiol Challenge

Clarified assessments will include adverse events related to estradiol administration as part of the estradiol challenge	8.0 REGULATORY AND REPORTING REQUIREMENTS
27 August 2015 Amendment 2 Version 4	
Title page: add clinicaltrials.gov number, update version number and date	Page 1
Footer – updated to current date	All pages of document
Update inclusion criteria to allow for newly diagnosed patients and some subjects with hepatic disease and to clarify inclusion criteria	3.1 Inclusion Criteria
Update exclusion criteria to remove exclusion for hepatic disease	3.2 Exclusion Criteria
Section 5.2 clarified that any subjects enrolled on study whose images are considered non-evaluable for any reason may be replaced	5.2 Patient Population
Section 5.3 created a larger window for time between administration of estradiol and repeat ^{18}F-FFNP imaging	Estradiol Challenge Test
26 October 2015 Study Renewal Version 5	
Update protocol title page and protocol footer to current date	All pages of document
24 November 2015 Amendment 3	
Update eligibility criteria to include surgery as option for determining response regardless of amount of time for neoadjuvant therapy	
01 April 2016 Amendment 4 Version 7	
Update protocol title page and protocol footer to current date for regulatory renewal	All pages of document
12 July 2016 Amendment 5 Version 8	
Update schema to remove metastatic this should have occurred with Amendment 3 when newly diagnosed patients were added as eligible to participate on study	STUDY SCHEMA
Update study schema to clarify that a minimum of 6 months of therapy or until response assessment should be planned	STUDY SCHEMA
Section 3.1 Inclusion Criteria: clarify that pathology for eligible breast cancer type can be from metastatic or primary pathology report	3.1 Inclusion Criteria
Section 5.3 Estradiol challenge add clarification statement "Whenever possible" to description of collecting blood sample for estradiol measurement.	5.3 Estradiol Challenge Test
Section 5.2 Clarify premenopausal subjects are eligible if ovarian suppression is planned.	5.2 Patient Population

Protocol Formatting remove extra spaces, duplicate words, add page breaks as needed for overall format of the protocol	As needed on all protocol pages
01-March-2017 Amendment 6 Version 9	
Update protocol title page and protocol footer to current date	Title Page
Added FDG-PET/CT to Study	SCHEMA
Updated table of contents	Table of Contents
Updated study objectives to include FDG-PET/CT imaging at baseline	Section 2.0 OBJECTIVES
Added FDG-PET/CT imaging to study related scans	5.2 Patient Population
Add FDG-PET/CT imaging to Section 6.0 Imaging parameters and Analysis	6.0 IMAGING PARAMETERS AND ANALYSIS
Add additional information FFNP-PET/CT imaging and use of port-a-caths	Section 6.3 18F-FFNP-PET/CT Imaging Parameters
Expected toxicities Section 6.6 added FDG-PET/CT	Section 6.0 Toxicities Related to 18F-FDG &-18F-FFNP PET/CT Imaging
Update study calendar section 7.0 to add FDG-PET/CT	Section 7.0 STUDY CALENDAR
Add FDG-PET/CT to regulatory and reporting requirements section 8.0	Section 8.0 REGULATORY AND REPORTING REQUIREMENTS
16 June 2017 Amendment 7 Version 10	
Fix typographical error 2 weeks is noted instead of 4 weeks for time between imaging sessions	Section 5.3 Estradiol Challenge Test
Clarify and update normal ranges for vital sign assessment section 6.0	Section 6.0 Vital Sign Assessments
05Feb2018 Renewal Version 11	
Update title page and footer of protocol to match date of renewal submission	Title Page
25May2018 Amendment 8 Version 12	
Clarify study schema standard of care metabolic imaging is acceptable in place of baseline FDG PET/CT scanning if it has already been performed.	SCHEMA
Revise time frame for which follow up chart review should occur	Section 6.8 Evaluation of Tumor Response Section 6.9 FOLLOW UP PROCEDURES
01February2019 Version 13 Annual renewal	
Update title page and footer of protocol to match date of renewal submission	Title Page

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SCHEMA

Standard of Care Diagnosis and Staging of Hormone-Sensitive Breast Cancer:

⬇

Scheduled to Receive:
Standard of Care Endocrine Therapy

⬇

Research Scan:
Pre-Treatment: ^{18}F-FDG PET/CT or confirmation of acceptable metabolic imaging done per SOC & ^{18}F-FFNP PET/CT Scan

⬇

Research (Estrdiol Challenge):
Total of 6 mg of estradiol administered (2 mg TID) over approximately 24 hours

⬇

Research Scan:
Repeat ^{18}F-FFNP PET/CT Scan

⬇

Planned Standard of Care Endocrine Therapy for a minimum of 6 months or response assessment is available
Standard Chart Review
For Clinical Response to Planned Treatment

Table of Contents

1.0 BACKGROUND

1.1 ENDOCRINE THERAPY AND BREAST CANCER

Breast cancer remains the leading cause of cancer mortality among women in Western countries. Current estimates suggest one in eight American women will be diagnosed with breast cancer during their lifetime. Distinct characteristic of breast cancer can be exploited to help determine the overall prognosis and the likelihood of response to specific therapy. It is well established that several factors including steroid receptors, peptide growth factors, oncogenes, and tumor suppressor genes, play crucial roles in determining tumor response to various endocrine therapies (ETs) and the development of resistance to these treatments in breast cancer (Weigel MT. Endocr Relat Cancer 2010;17:R245-262). The majority of patients with breast cancer have disease that is estrogen-receptor positive (ER+), and in general this form of breast cancer is a less aggressive disease than estrogen receptor-negative (ER-) disease. ER+ disease occurs more commonly in postmenopausal women and is characterized by longer disease-free and overall survival. Even with metastatic disease, the median survival in patients with estrogen-receptor-positive (ER+) tumors is three times longer than in those with estrogen-receptor-negative (ER-) breast cancer (Keen JC. Cancer 2003;97:825-83).

In treating metastatic breast cancer, the hormone-receptor status directs systemic therapy. ER+ disease will respond to first-line endocrine therapy (ET) in 55-60% of patients (Goldhirsch A. Ann Oncol. 2002;13(suppl 4):61–68). Nevertheless, nearly half of the patients with ER+ disease still fail to respond to ET. Because ER is a key transcription factor for the production of the progesterone receptor (PgR), the presence of PgR in ER+ cells is thought to indicate that the estrogen-response pathway is functional in these tumors and, thus able to mediate the benefits of ETs. The In fact, the presence of PgR in ER+ disease does presence of PgR increases the likelihood of hormone responsiveness to some degree (Keen JC. Cancer 2003;97:825-833). The presence of the progesterone receptor (PgR) increases the likelihood of hormone responsiveness to some degree (Keen JC. Cancer 2003;97:825-83). Progesterone-receptor negative (PgR–) tumors are less responsive to therapy, perhaps suggesting that PR may be necessary for positive therapeutic outcome with ET. Alternatively, because ER is a key transcription factor for the activation of PgR, lack of PgR expression in these ER+/PgR– cells also could suggest that estrogen response pathway may not be functional in these tumors. Only a small fraction of tumors are ER– and progesterone-receptor positive (PgR+) (< 5%) and they demonstrate an intermediate response to ET (Keen JC. Cancer 2003;97:825-83). It has been suggested that PgR loss may be a surrogate marker of excessive growth factor receptor signaling and consequently poor response to ET (Osborne CK. Breast. 2005 Dec;14(6):458-65). It is also possible that low PgR levels in ER+ disease might indicate that estradiol levels are insufficient to induce through ER significant PgR levels or that ER is not functional.

Challenges in selecting the best treatments for breast carcinoma.
ER positivity by immunohisto¬chemistry (IHC) is a prerequisite for ET. However, up to 50% of patients with ER+ breast cancer do not benefit from hormonal therapy as a result of intrinsic or acquired resistance mechanisms (EBCTCG. The Lancet 2011, 378(9793):771-784; Dowsett M. J Clin Oncol 2010, 28(3):509-518; Mouridsen H. J Clin Oncol 2003, 21(11):2101-2109). In the early-stage setting, multi-gene assays such as Oncotype Dx, Mammaprint and Prosigna, have

been developed as prognostic tools to assess the need for chemotherapy; however, their value in predicting ET efficacy has not been established. While over 20% of patients with ER+ breast cancer develop recurrent disease because of ineffectiveness of hormonal therapy (Cuzick J. The Lancet Oncology 2010, 11(12):1135-1141), a large number of patients suffer from treatment-related adverse events leading to treatment discontinuation (Crew KD. J Clin Oncol 2007, 25(25):3877-3883). For patients with ER+ metastatic breast cancer, ET is often offered as the front-line treatment; however, this approach delays appropriate treatment in up to 50% of patients in whom ET is ineffective. In addition, ER status can change over time and be variable among metastatic sites within the same patient. It is not practical, however, to biopsy all sites of metastatic disease to characterize fully the ER status of a patient's tumor burden. Our proposed PET-imaging strategy, described below, would allow for functional assessment of the ER pathway non-invasively and in all macroscopic tumor foci, and, if successful, has the potential to improve treatment decisions in patients with ER+ breast cancer.

In vitro assessment of hormone-responsive breast cancer: Immunohistochemical (IHC) assays of ER and PgR have numerous shortcomings; better predictive assays are needed. Selection of ET is based on hormone receptor status of the breast cancer, which is routinely assessed by in vitro IHC assays that have increasingly replaced quantitative radioligand binding assays. IHC determinations of hormone receptor status in breast carcinoma are equally sensitive, and more specific than biochemical determinations by ligand binding (Rhodes A. J Clin Pathol 2000;53:292-301; Hammond ME. Arch Pathol Lab Med 2010;134:e48-72.). However, while the results of IHC assays provide some predictive value in selecting the best follow up therapies, as noted above, even in ER+ disease, response rates to ETs are only approximately 50%. Furthermore, IHC assays of ER and PgR have a number of other shortcomings: (a) Most notably, they provide limited information about the functional status of the receptors and the responsiveness of tumor to ET, as described above. (b) The evaluation criteria to establish the positivity of ER and PgR are also not uniform in different laboratories, and widely ranging cutoff values for distinguishing positive from negative results have been used in IHC assays for both receptors. For example, a positive result can be considered the presence of greater than 1% or 5%, or even 10%, IHC-positive cells (Elledge RM. Int J Cancer 2000;89:111-117). (c) A recent systematic review of the literature on the use of IHC for evaluation of ER and PgR found that up to 20% of current IHC test results worldwide may be inaccurate (false negatives or false positives) (Hammond ME. Arch Pathol Lab Med 2010;134:e48-72). (d) Also, tumors are heterogeneous, and fine needle or core biopsies may miss important regions of the tumor. (e) Finally, in recurrent or metastatic breast cancer, the ER or PgR status of the lesions may not always be the same as that of the original primary tumor. Indeed, the receptor status of recurrent or metastatic disease may be more predictive of response to ET. However, because metastatic lesions often are not amenable to biopsy and because the biopsy of multiple lesions is impractical, the receptor status of individual lesion(s) cannot be easily determined.

1.2 IMAGING ER and PR in BREAST CANCER USING PET: FES and FFNP

Non-invasive assays of prognostic and predictive biomarkers by PET: advantages of in vivo assessment of ER and PgR. The in vivo measurement of the ER and PgR expression in breast cancer could offer several advantages over current in vitro methods. These include (a) assessing ER and PgR expression of the entire tumor volume rather than just a part of the tumor (addressing the intrinsic heterogeneity of receptor expression and limited sampling by needle biopsy), (b) directly assessing the binding of the receptors to the imaging agent, a hormone analogue, rather than their antigenic properties as measured by IHC, and (c) evaluating the effects of therapy on receptor expression of the tumor, as ER and PgR expression may change during therapy. Finally, (d) in vivo imaging can simultaneously assess the receptor expression of primary and metastatic sites, which may vary across lesions within any given patient.

Positron emission tomography (PET) is a highly sensitive functional imaging technique that provides high-resolution images and enables subnanomolar concentrations of receptor targets to be quantified. PET using radiolabeled agents is a novel option for non-invasive identification of the presence of specific targets throughout the body, tracing and quantifying the distribution of imaging agent binding to the target and ultimately helping to better understand the in vivo behavior and effectiveness of ET of individual patients. Significant efforts have been made to develop imaging agents labeled with positron-emitting radionuclides for noninvasive evaluation of ER and PgR expression, and localization of ER and PgR-overexpressing tumors. However, while several radiopharmaceuticals for imaging ER-positive lesions have been developed, including [^{18}F]-fluoroestradiol (FES), which is now widely used in many medical centers worldwide, the development and evaluation of PgR-imaging based tracers has lagged behind (See below) (Linden, HM & Dehdashti F. Semin Nucl Med. 2013 Jul;43(4):324-9).

The focus of this project is to investigate the use of PgR-PET to improve selection of those patients with advanced breast cancer who will benefit from endocrine therapies.

Assessment of functional status of ER with FES-PET. To assess in vivo ER availability and functional status, efforts have been ongoing for over two decades to identify and evaluate radioligands with high affinity and selectivity for binding to ER and with properties suitable for imaging. Several steroidal and non-steroidal estrogens labeled with 77Br, 75Br, 123I, and 18F have been synthesized (Katzenellenbogen JA. J Nucl Med. 1995 Jun;36(6 Suppl):8S-13S; ; Jonson SD. Q J Nucl Med. 1998 Mar;42(1):8-17). One of the most promising positron-emitting radiolabeled estrogen analogs identified is FES. This radioligand has high specific activity, high selective ER binding in vitro, and high affinity for ER+ target tissues (e.g., uterus and mammary tumors) in animal models (Kiesewetter DO. J Nucl Med. 1984 Nov;25(11):1212-21; Mathias Ci., Intl Radi4ppl Instiwn [B] 1987;14:15â; Brodack JW., Int J Rad Appl Instrum A. 1986a;37(3):217-21; Brodack JW J Nucl Med. 1986b May;27(5):714-21). We and others have utilized FES to assess the functional status of tumor ERs in women with breast cancer, and we have shown that tumor uptake of FES correlates with ER levels measured in vitro and may be more predictive of response to hormonal therapy than knowledge of the tumor ER status (Mintun MA Radiology 169:45-48, 1988,; Mortimer JE Clin Cancer Res 2:933-939, 1996; Linden HM. . J Clin Oncol. 2006 Jun 20;24(18):2793-9; Linden HM. Clin Cancer Res. 2011 Jul 15;17(14):4799-805; Peterson LM1. Mol Imaging Biol. 2014 Jun;16(3):431-40; Currin El. Curr Breast Cancer Rep. 2011 Dec;3(4):205-211). FES is currently being studied in a number of clinical research centers in the US (most notably by us at Washington University in St. Louis, but also at the University of Washington, Memorial Sloan Kettering Cancer Center and Harvard Medical School) and internationally (Korea, Japan, Europe) to evaluate its predictive value in selecting patients for different endocrine therapies. Through this work, FES has been shown to have a high negative-predictive value (NPV), i.e., absence of FES uptake in tumor means that response to ET is unlikely, but its positive-predictive value (PPV) has been limited in identifying patients who are likely to respond to ET.

As stated above, ER status may be discordant within the same patient; thus, a single biopsy may not be representative of the ER characteristics of the entire tumor burden in the patient. Several studied evaluated within patient heterogeneity of FES uptake (Mortimer JE Clin Cancer Res 2:933-939, 1996; Kurland BF J Nucl Med. Oct 2011; 52(10): 1541–1549 and Yang Z Clinical Breast Cancer, Vol. 13, No. 5, 359-63 2013). We found heterogeneity (discordance) in FES uptake in 4 of the 17 (24%) patients with breast cancer who had multiple metastatic lesions; each patient had a single discordant site (Mortimer JE Clin Cancer Res 2:933-939, 1996). Yang et al. reported discordance in FES uptake in 9 of 32 (28%) of their patients with metastatic breast cancer (Yang Z Clinical Breast Cancer, Vol. 13, No. 5, 359-63 2013). The difference was 8.2-fold in FES uptake among lesions within the same individual. In a subgroup analysis of the patients who had prior ET, 9 of the 24 patients (37.5%) showed within patient heterogeneity in FES uptake. It is possible that ER heterogeneity plays an important role in determining response to ET in those with substantial within patient heterogeneity in receptor expression. As a noninvasive tool, PET as a noninvasive tool has the potential to provide this important information on disease heterogeneity, which may be crucial in selection of the mode of therapy in the patients with metastatic breast cancer, an aspect that we plan to explore in Specific Aim 2 in this project.

PgR imaging with PET in breast cancer patients: ^{18}F-FFNP, an imaging agent of great promise. As stated above, the combination of ER and PgR expression is a stronger predictor of response to ET than either alone. The search for a more suitable progesterone-based imaging compound was continued by the Katzenellenbogen group, and they have described several new F-18 labeled radioligands. One of these, 21-[^{18}F]fluoro-16α,17α-[(R)-1'-α-furylmethylidene)dioxy]-19-norpregn-4-ene-3,20 dione (^{18}F-FFNP), a radioligand with high affinity and selectivity for PgR and improved imaging characteristics, has been developed. ^{18}F-FFNP showed very marked and selective uptake in target tissues in rodents. In addition, only low levels of ^{18}F-FFNP accumulated in the liver and fat, because its decreased lipophilicity translated into low in vivo non-specific binding. This compound is considered to be the most promising progestin derivative for PET imaging (Kochanny et al., J Med Chem. 1993; 36(9):1120-7; Buckman et al., J Med Chem. 1995; 38(2): 328-37; Kym et al., J Med Chem. 1993; 36(9):1111-9; Vijaykumar et al., J Org Chem. 2002; 67(14):4904-10).

A first in human study that evaluated the safety and dosimetry of ^{18}F-FFNP has been completed at Washington University. Twenty patients with 22 primary breast cancers (2 patients each had two cancers in different quadrants of the same breast) were evaluated. We showed that a significantly higher tumor uptake ($p = 0.001$) in PgR+ than PgR- breast cancer. The study also showed that ^{18}F-FFNP-PET imaging is a safe method for evaluating tumor PgR non-invasively in patients with breast cancer (Dehdashti et al, 2012 J Nucl Med. 2012 Mar;53(3):363-70). In addition, human radiation doses calculated from the PET images indicated an effective dose of 0.02 mSv/MBq, a value that is comparable to that reported for 16α-[^{18}F]fluoro-17α-estradiol (^{18}F-FES) (0.022 mSv/MBq), an estrogen-receptor imaging tracer (see below).

1.3 ASSESSMENT of ER FUNCTION WITH PET:

ER and PgR IHC assays, and clinical flare have very limited predictive value. The initial choice of agents for treating advanced disease is based on the status of the ER; typically: Patients with hormone -sensitive receptor-positive disease are treated with hormone manipulation, whereas those with hormone -resistant receptor-negative disease receive chemotherapy. Although most breast cancer is ER+, ET is underutilized in this country in favor of more toxic chemotherapy regimens. In part, this is because ETs do not always succeed, even in ER+/PgR+ cancers,. Also, many oncologists believe that response to chemotherapy occurs more rapidly and is easier to assess than the response to ETs.

A so-called "clinical flare reaction" occurs in 5-20% of women with breast cancer who receive certain hormonal therapies. Within 7-10 days after starting ET (particularly with tamoxifen), patients who experience a flare reaction have subjective and objective findings suggesting disease progression. It is postulated that this transient flare reaction is caused by initial agonist effects when tamoxifen levels are low; however, with continued treatment, tamoxifen becomes antagonistic, frequently causing subsequent tumor regression in a patient who had an initial flare (Vogel J Clin Oncol 1995; 13:1123–1128). Thus, the flare reaction, when it occurs, is an indicator of functioning ERs and is a predictor of therapeutic responsiveness, as 80% of these patients will respond with continuation of the hormonal agent (Vogel J Clin Oncol 13:1123–1128). Clinically, however, it is difficult to distinguish a flare reaction from disease progression and this, as well as its low frequency, makes it an insensitive and unreliable predictive index of ET response.

A hormone-challenge paradigm using tamoxifen or estradiol: an indirect assessment of ER function by a "metabolic flare". We studied women with advanced hormone-sensitive breast cancer by serial PET imaging with 18F-fluorodeoxyglucose (FDG) and FES before and 7-10 days after tamoxifen therapy was initiated to investigate whether the metabolic correlates of a subclinical flare reaction, due to the initial agonist effects of the drug, could be documented by functional imaging (Mortimer, JE. J Clin Oncol. 2001; 19(11):2797-803). This study demonstrated that PET provides unique information about tumor response at the biochemical level early during therapy (within 10 days) that could be used to predict ultimate therapeutic response (Dehdashti, F. Eur J Nucl Med. 1999 Jan;26(1):51-6, Mortimer, JE. J Clin Oncol. 2001; 19(11):2797-803). Our findings supported our hypothesis that tumor receptor levels and hormone-induced metabolic flare reactions could be assessed by imaging in vivo with FES-PET and FDG-PET. The most important single predictors of response to tamoxifen were high baseline FES uptake (P=0.0007) and an increase in FDG uptake after tamoxifen (P=0.0002). The latter measure gave PPV and NPV of about 90%, which considering that all patients were ER+ by IHC assays, represented significantly improved predictive accuracy, notably obtained after only 10 days of treatment. This study, which concluded some time ago, included only patients who received tamoxifen as the initial treatment for their advanced disease. Now, many women who present with metastatic disease have already been treated with tamoxifen in the adjuvant setting. In such patients, additional second-line and third-line hormonal therapies include aromatase inhibitors (AIs) and the full estrogen antagonist, fulvestrant.

Because the mechanisms of action of these new endocrine therapy agents are different than that of tamoxifen, they do not typically cause clinical flare. Accordingly, we investigated the induction of a "metabolic flare reaction" using a brief treatment with estradiol, a more potent estrogen than tamoxifen, seeking to find a more robust response. Once again, we found that both baseline tumor FES uptake and metabolic flare, assessed by serial FDG-PET, after a 1-day estradiol challenge, were predictive of responsiveness to endocrine therapy in ER+ breast cancer (Dehdashti F. Breast Cancer Res Treat. 2009 Feb;113(3):509-17). We found that with an increase in tumor FDG uptake ≥ 12% as the criterion for defining estradiol-induced metabolic flare based on ROC curve analysis, the PPV for response to endocrine therapy was 100% (all 15 of such patients responded) and the NPV was 94% (only 2 of 36 such patients responded). The baseline FES uptake (using a cutoff SUV of ≥ 2 as the criterion for defining functional ER based on our prior experience) had PPV and NPV for response to therapy of 50% (12 of 24 FES+ patients responded) and 81% (5 of 27 FES– patients responded), respectively. Thus, metabolic flare assessed by FDG-PET was again a stronger predictor of response to ET than a direct measure of ER level by FES-PET.

Subsequently, we studied another group of patients with hormone-sensitive metastatic breast cancer treated with an AI, with at least 24 weeks of progression-free survival, or relapse after two or more years of adjuvant AI, again using serial FDG-PET and estradiol challenge. An estradiol stimulated increase in FDG uptake of ≥ 12% (prospectively defined from the prior study) was predictive of response (PPV of 80%; 95% CI: 61%–92%). (Ellis MJ. JAMA 2009 Aug 19;302(7):774-80). This compares with PPV of 100% on the prior study.

While the results from this FDG-PET hormone challenge test were promising, the increase in the SUV for FDG after estradiol was relatively modest in responders, with increases rarely exceeding 40%. Consequently, many values lie within 5% of the cutoff, indicated by the fact that 25% of all the values lie within the gray area. Thus, this hormone-challenge test lacks the level of robustness needed to be truly useful at different clinical sites. Consequently, to make the best prediction of potential benefit from ETs, there is a need for a more sensitive and robust test for the functional status of ER in breast tumors by PET

A direct assessment of the functional status of ER with FFNP-PET as a predictor of response to ET. PgR is a gene highly regulated by ER at the RNA and protein level, and the presence of PgR in ER+ breast cancer was proposed to indicate that ER was functional and therefore that the likelihood of benefit from endocrine therapy would be greater. While reasonable, this idea has not been uniformly accepted, and PgR levels in breast cancer are often not considered in therapy decisions (Davies C, Godwin J, Gray R, et. al. Lancet. 2011, 378, 771-784. PMCID: 3163848). The conclusion that PgR levels are not of predictive importance, however, represents a serious oversight: One should expect PgR levels to be high only if ER is functional and the stimulating hormone, estradiol, were present at sufficient levels. PgR assay results, however, are not controlled for endogenous estradiol levels, which in most cases (post-menopausal patients) would be very low! Thus, we believe that using ^{18}F-FFNP -PET to measure whether PgR levels in tumors change upon estrogen stimulation will prove to be a very sensitive way to demonstrate that tumor ERs are functional and thus likely to mediate response to ETs

We have established the efficacy of this "hormone-challenge test for functional ER" in new mammary cancer models derived from STAT1-/- mice (Fowler A. J Nucl Med. 2012; 53:1119-26). In ovary-intact mice, SSM2 tumors, which are ER+ but ET non-responsive, show no decrease in PgR (PgR-A and PgR-B) levels upon antiestrogen with Fulvestrant treatment, whereas SSM3 tumors, which are ER+ and ET responsive, show a great reduction in PgR levels. More notably, in the SSM3 ER+/ET-responsive tumors, both a large decrease in ^{18}F-FFNP uptake after Fulvestrant treatment and a large increase in ^{18}F-FFNP uptake are observed after estradiol. The average tumor-muscle (T/M) ratios increased from 3.6 for the untreated mice to 6.9 in SSM3 mice after 24 hours of estrogen stimulation. Therefore, an up or down change in ^{18}F-FFNP T/M ratio in mammary tumors accurately predicted sensitivity to estrogen addition or deprivation therapy, respectively, and was able to distinguish between ER+/endocrine-responsive and ER+/endocrine non-responsive disease.

More recently, Chan et al. (manuscript in preparation) used the same model to follow the decrease in ^{18}F-FFNP mammary tumor uptake in response to estrogen deprivation therapy by ovariectomy, which reduces tumor PgR levels. Notably, in this study, they compared PET imaging using ^{18}F-FDG (to measure glucose uptake), ^{18}F-FES (to measure ER levels), or ^{18}F-FFNP (to measure PgR levels) to see which probe would best predict response to estrogen deprivation therapy. Uptake in endocrine-sensitive and -resistant mammary tumors (both of which were ER+) was measured by PET in mice before ovariectomy, and on days 3 and 4 after this form of estrogen deprivation therapy. Specificity of ^{18}F-FFNP uptake in ER+ mammary tumors was determined by competition assays using unlabeled ligands for PgR and confirmed by IHC. The levels of ^{18}F-FES and FDG tumor uptake remained unchanged in endocrine-sensitive or resistant tumors after estrogen deprivation therapy compared to those at pre-treatment. By contrast, estrogen deprivation therapy led to a reduction in PgR expression and ^{18}F-FFNP uptake in endocrine-sensitive tumors, but not in endocrine-resistant tumors, as early as 3 days post-treatment, importantly demonstrating that *PgR-PET provides a more sensitive and robust measure of tumor response than* ^{18}F-*FES- or* ^{18}F-*FDG-PET*.

This type of hormone-challenge paradigm was validated by us in human breast cancer patients, as described above, using ^{18}F-FDG-PET to measure changes in tumor metabolism after estradiol, and although it was highly predictive of ET response, the ^{18}F-FDG changes were mostly quite small and were less predictive of response to ET in patients who had been heavily treated with ET (Dehdashti F. Breast Cancer Res Treat. 2009 Feb;113(3):509-17; Ellis MJ. JAMA 2009 Aug 19;302(7):774-80). Because PgR is more acutely regulated by estrogen, it should offer a much greater dynamic range of response.

2.0 OBJECTIVES

Our hypothesis is that change in ^{18}F-FFNP uptake following 1-day of estradiol is a strong predictor of response to ET in patients with hormone-sensitive (ER+/± PgR+)/HER2- breast cancer. We believe the increase in tumor PgR after 1-day estradiol will be more reliable than a decrease after estrogen deprivation, particularly because most breast cancer patients are postmenopausal and basal PgR levels will be low due to the low endogenous, menopausal estrogen levels. Thus, we propose to use a hormone-challenge test for assessment of functional

ER based on the change in ^{18}F-FFNP uptake before and after a 1-day estradiol challenge in patients with ER+ metastatic breast cancer. We expect that the changes in ^{18}F-FFNP uptake to be a highly reliable means to predict a favorable response of ER+ breast cancer to ET. We propose to study patients with biopsy-proven new diagnosis of ER+ metastatic/recurrent breast cancer who are going to be treated with ET (tamoxifen, AI agents or faslodex) according to standard of care.

2.1. Evaluate whether the change in tumor uptake of ^{18}F-FFNP following a 1-day estradiol challenge differs among patients who respond to ET versus those who do not respond.

2.1.1 Determine the optimum cutoff value for change in tumor ^{18}F-FFNP uptake after estradiol to distinguish responders from nonresponders.

2.1.2 Evaluate whether the change in tumor uptake of FFNP can identify patients with hormone-sensitive disease who will respond to ET with greater sensitivity and selectivity than tumor PgR measured by IHC.

2.2 Evaluate the heterogeneity of tumor ^{18}F-FFNP uptake at baseline and after estradiol challenge in patients with multiple metastatic foci.

2.2.1. Explore whether response is related to ^{18}F-FFNP uptake heterogeneity. To assess the heterogeneity at the baseline, a comparison with FDG-PET/CT will be performed to map the metabolically active disease. Once the metabolically active disease is determined, FDG+/FFNP- will be considered as discordant lesions. At follow-up, the baseline FFNP-PET/CT will be correlated with follow-up FFNP-PET/CT to assess heterogeneity in response to estradiol.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

1. Patient must be postmenopausal defined as meeting one or more of the following:
   a. Age ≥ 60 years
   b. Amenorrheic for at least 12 months
   c. Surgically sterile- having undergone bilateral oophorectomy,
   d. FSH laboratory test level in postmenopausal range according to institutional standards (note FSH lab test must be ordered as standard of care to determine best treatment option and should not be ordered simply to confirm eligibility to this study)

   e. OR Pre-menopausal for whom standard ET is planned with ovarian suppression (imaging on study should be completed prior to start of ovarian suppression)
2. Patient must have histological or cytological confirmed breast cancer and fall into one of the following categories:
   a. New diagnosis with plans for at least 6 months of neoadjuvant ET or any amount of neoadjuvant ET if surgery is planned as this will be used for response assessment .
   b. Patients with newly diagnosed metastatic breast cancer or patient with known metastatic disease who has progressed while on therapy (no washout period is needed if the patient was treated with AIs or chemotherapy, but 2 months washout period is needed if the patient was treated with tamoxifen) who are going to be treated with ET.
3. Patient must have any one of the following types of breast cancer (primary or metastatic): ER+/PgR+/HER2– or ER+/PgR–/HER2–.
   a. ER+ is defined as Allred score of at least 4 and greater.
   b. PgR+ is defined as Allred score of at least 4 and greater.
   c. IHC is the primary assay methodology for HER2. HER2– refers to HER2 of 0, 1+ by IHC or negative by fluorescence in situ hybridization (FISH)
4. Patient must have at least one measurable lesion according to RECIST 1.1 by radiological evaluation (ultrasound, mammography, MRI, CT, PET) or physical examination.

a. Patients with evaluable osseous metastasis that are lytic or mixed lytic-sclerotic are eligible.
b. Patients with hepatic lesions may be eligible provided the location of the lesion is peripheral or not too close to hepatic ducts. Decision on hepatic lesion eligibility will be made by the principal investigator or sub-investigator after careful review of all available imaging to ensure evaluation of the lesion will not be obscured by normal hepatobiliary excretion of ^{18}F-FFNP.

5. Patient must be able to understand and willing to sign a written informed consent document.
6. Prior chemotherapy or endocrine therapy is allowed
7. The patient must have an ECOG performance status of 0-2 or, based on the judgment of the treating medical oncologist, can tolerate imaging and at least 6 months of ET
8. The patient should have a life expectancy of > 6 months.

3.2 Exclusion Criteria

1. Patient with other invasive malignancies, with the exception of non-melanoma skin cancer or cervical carcinoma in-situ, who had (or have) any evidence of the other cancer present within the last 5 years
2. Unable to tolerate up to 60 min of PET imaging per imaging session.
3. Patients with non-measurable non-evaluable lesions such as pleural effusion are not eligible to participate.

4. Patients with vertebral lesions that, in the opinion of the Principal Investigator and the treating medical oncologist, pose an imminent risk for cord compression.

3.3 Inclusion of Men and Minorities

Because breast cancer occurs most often in women, and the receptor status of breast cancer in women has been studied more often, men will not be eligible for this trial. The trial is open to members of all races and ethnic groups and participation will be encouraged.

4.0 PATIENT REGISTRATION

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility collecting the information listed below:

1. The registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center Database

All patients must be registered through the Siteman Cancer Center database.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 METHODS

5.1 Study Design

A single center, open-label, baseline controlled diagnostic imaging study designed to assess the predictive value of ^{18}F-FFNP-PET/CT imaging women who complete an estradiol challenge test.

5.2 Patient Population

Fifty postmenopausal women OR Pre-menopausal for whom standard ET is planned with ovarian suppression (imaging on study should be completed prior to start of ovarian suppression) with newly diagnosed, metastatic or recurrent breast cancer which is ER+ disease (ER+/PgR+/HER2–, ER+/PgR–/HER2–) will undergo one FDG PET/CT scan to map the metabolically active disease plus two ^{18}F-FFNP-PET/CT; one prior to estradiol challenge test and a second one immediately following one day of estradiol challenge test (2 mg tid). All scans will be completed prior to the start of standard of care ET. Patients who have had an FDG-PET/CT scan (or other functional studies such as bone scan in patients with bone dominant disease or a contrast CT scan for hepatic dominant disease) done within 8 weeks of study entry may not need to repeat the scan at the discretion of the Principal Investigator and Co-Investigator Medical oncologists. Any subject whose imaging is deemed not evaluable for any reason or any subject who does not complete both FFNP-PET/CT imaging sessions for any reason may be replaced on study. The study ID assigned to the subject being replaced will not be re-used.

5.3 Estradiol Challenge Test

The estradiol challenge test will consist of administering a total of 6 mg of estradiol dosed orally as three 2 mg tablets with each tablet being administered approximately 8 hours apart and within a 24 hour period. This estradiol medication will be provided to the patient by the study. Dosing will be such that the third dose of estradiol will be administered at a minimum 8 hours (8 ± 2 hours) and a maximum 48 hours prior to the scheduled injection of ^{18}F-FFNP for the repeat ^{18}F-FFNP-PET/CT imaging session. Timing of the estradiol administration is critical so that the effect of estradiol will be captured within the imaging session. The first dose of estradiol can be administered as soon as immediately following the baseline ^{18}F-FFNP-PET/CT imaging session with the post estradiol ^{18}F-FFNP -PET/CT imaging session being scheduled for the following day. No more than a maximum of 4 weeks should occur between the baseline and the post estradiol challenge ^{18}F-FFNP -PET/CT imaging sessions. A subject specific calendar will be given to each participant that includes dates and times for estradiol administration and follow up ^{18}F-FFNP-PET/CT imaging session. Whenever possible, blood will be obtained for measurement of the serum estradiol level, using a high-sensitivity radioimmunoassay (Herting MM. BJ. Cereb Cortex. 2012 Sep;22(9):1979-92) before injection of ^{18}F-FFNP at the baseline and after estradiol challenge before the 2nd ^{18}F-FFNP injection to document that it increased appropriately.

5.4 Risks Related to Estradiol Challenge Test.

Side effects from taking a total 6 mg of estradiol over a 24 hour period are expected to be minimal. Subjects may experience nausea from a dose this small. When estradiol is administered in much higher doses as therapy and given daily over a longer period of time the following side effects have been reported: nausea and or vomiting; bone pain, breast tenderness and/or enlargement; vaginal bleeding, tiredness, changes in skin color; insomnia (difficulty falling or staying asleep); yeast infections, increase or decrease in vaginal secretions, and headaches

6.0 IMAGING PARAMETERS AND ANALYSIS

All research imaging studies will be performed using the CTI/Siemens Biograph 40 PET/CT scanner. The Biograph 40 is a 4-ring PET scanner made up of a multi-LSO-detector ring system with 3D acquisition and reconstruction and 109 image planes with an extended 21.6 cm axial field of view, enabling the detection of 78% more photons (compared with a conventional-field-of-view scanner). The scanner features high resolution (less than 5mm in transaxial and axial dimensions) with Pico 3D ultra-fast electronic for decreased dead-time and high signal-to-noise ratio.

All patients will undergo routine clinical staging as dictated by the treating medical oncologist or surgeon. The results of the PET studies will not be provided to the patient or the treating oncologist unless the CT images demonstrate an unsuspected, potentially life-threatening abnormality that warrants further investigation and/or urgent therapy (e.g., a mass impinging on the spinal cord seen on the CT images).

If performed for research purposes, the results of the ^{18}F-FDG-PET/CT scan will be fully interpreted and reported to the medical record following the same procedures as if done as a standard of care examination.

6.1 Drug Preparation

6.1.1 ^{18}F-FFNP

^{18}F-FFNP will be prepared using an adaptation of a published procedure (Buckman BO J Med Chem. 1995;38(2):328-37; Kym PR J Med Chem. 1993;36(9):1111-9 ; Vijaykumar D J Org Chem. 2002;67(14):4904-10) . The diastereomerically pure 21-mesylate, endo-9a precursor was reacted with non–resin-treated [^{18}F]-fluoride, Kryptofix222 (Aldrich), and potassium carbonate in acetonitrile at 85C for 5 min. The reaction mixture was prepurified by passing through a silica light SepPak (Waters), followed by reversed-phase high-performance liquid chromatography purification. ^{18}F-FFNP was extracted from the high-performance liquid chromatography mobile phase using solid-phase extraction and was reconstituted in 10% ethanol in saline. Starting from 11.1 GBq (300 mCi) of 18Ffluoride and using 0.4 mg of potassium carbonate and prompt work-up to avoid the decomposition of the acid- and base-labile ^{18}F-FFNP, we produced 0.74–1.11 GBq (20–30 mCi) of ^{18}F-FFNP at the end of synthesis (90 min). The final formulation of ^{18}F-FFNP is stabilized in ethanol or saline. Non–resin-treated ^{18}F-fluoride was used to achieve high specific activity. The specific activity was measured by high-performance liquid chromatography to be 185–740 GBq (5,000–20,000 mCi)/µmol, and the effective specific activity was measured by receptor binding assay to be up to 740 GBq (20,000 mCi)/µmol.

6.1.2 ^{18}F-FDG

FDG will be prepared and distributed under the Washington University Cyclotron Facility's ANDA for FDG.

6.2 18F-FDG-PET/CT Imaging Parameters

If performed for research purposes, subjects will undergo standard oncologic ^{18}F-FDG-PET/CT imaging that includes the base of the skull to upper thighs (or extended to include metastatic disease in the lower legs, or possible brain metastasis, if applicable) imaging.

Subjects will be asked to fast for a minimum of 4 hours with only plain water to drink during the fasting period. On the day of the scheduled scan a small IV catheter will be placed in an upper extremity arm vein (preferably contralateral to the patient's breast cancer) to allow for injection of FDG. Prior to injection a small sample of blood (less than 1 teaspoon) will be obtained for glucose measurement. Injection of FDG will proceed if blood glucose, if 200 mg/dL or less or with approval of the PI or nuclear medicine co-investigator (authorized user), if blood glucose is greater than 200 mg/dL.

FDG dose will be based upon weight according to the standard nuclear medicine imaging protocol with the average dose injected 15 mCi. Imaging will occur 50-70 minutes post FDG injection. Subjects should be asked to rest and remain quiet, comfortable, and warm during the uptake phase. To avoid brown fat uptake the use of warm blankets is encouraged. IV or oral hydration (up to 500 ml unless subject has known fluid restrictions) is also encouraged during the uptake period.

Immediately prior to imaging, subjects will be asked to empty the bladder in the restroom. The subject will be placed supine on the imaging table with arms resting above the head or secured comfortably by the side of the body. A spiral CT scan for attenuation correction will be obtained from the skull through the upper thighs. The CT will consist of a 10-20 second topogram for determining correct anatomical positioning followed by a spiral CT at a maximum of 50 mAS. Care dose will be calculated for each scan and the care dose imaging parameters will be used if less than the maximum 50 mAs is calculated. CT imaging will be acquired with a standard 120 kVp. Average spiral CT scan time is 15-30 seconds. Scans are acquired using a 5 mm slice thickness.

Immediately after the attenuation CT scan and approximately 30 minutes after injection of FFNP, emission imaging will be obtained (2-5 minutes per bed position adjusted as needed based on subject height, weight, and injected dose).

6.3 ^{18}F-FFNP-PET/CT Imaging Parameters

^{18}F-FFNP-PET body imaging will includes the base of the skull to upper thighs (or extended to include metastatic disease in the lower legs, or possible brain metastasis as applicable) imaging. Site of scan can also be modified based on information obtained from FDG-PET/CT imaging.

There are no eating or drinking restrictions for ^{18}F-FFNP-PET imaging. On the day of the scheduled scan a small IV catheter will be placed in an upper extremity arm vein (preferably contralateral to the patient's breast cancer) to allow for injection of ^{18}F-FFNP. NOTE: FFNP is sticky and injection through port-a cath or extended amounts of IV tubing should be avoided whenever possible. Baseline vital signs consisting of blood pressure, heart rate, breathing rate and temperature will be obtained prior to the injection of ^{18}F-FFNP. A maximum dose 10 mCi (dose range 7-10 mCi) of ^{18}F-FFNP will be injected into the established IV line. The dose will be followed with a normal saline flush of 10-30 ml. Subjects will be asked to rest comfortably in the injection room for approximately 30-40 minutes while ^{18}F-FFNP circulates in the body (warm blankets and oral hydration is encouraged). Approximately 10-30 minutes after injection and during this resting period vital signs will be taken to assess for any post injection changes.

Immediately prior to imaging, subjects will be asked to empty the bladder in the restroom. The subject will be placed supine on the imaging table with arms resting above the head or secured comfortably by the side of the body. A spiral CT scan for attenuation correction will be obtained from the skull through the upper thighs. The CT will consist of a 10-20 second topogram for determining correct anatomical positioning followed by a spiral CT at a maximum of 50 mAS. Care dose will be calculated for each scan and the care dose imaging parameters will be used if less than the maximum 50 mAs is calculated. CT imaging will be acquired with a standard 120 kVp. Aveage spiral CT scan time is 15-30 seconds. Scans are acquired using a 5 mm slice thickness. Immediately after the attenuation CT scan and approximately 30 minutes after injection of FFNP, emission imaging will be obtained (2-5 minutes per bed position adjusted as needed based on subject height, weight, and injected dose). The ^{18}F-FFNP dose and the scans parameters will be kept as close as possible for both ^{18}F-FFNP-PET/CT scans in each patient.

At the end of the imaging session, subjects will be encouraged to void and post imaging vital signs will be assessed prior to discharge.

6.4 Vital Sign Assessments

All vital signs will be recorded on the case report form. Vital signs may be obtained with the subject in the supine or upright position. Care will be taken to obtain subsequent recordings with the subject in the same position (supine or upright). Although allergic or other immediate adverse reactions are not anticipated, subjects will be monitored for at least 30 min post injection in an area where emergency equipment is available. Vital signs will be obtained pre-injection, within 30 min post injection, and at the completion of each imaging session. Vital signs will include the following: heart rate, systolic blood pressure, diastolic blood pressure, respiratory rate, and body temperature. Changes in vital sign assessment will be determined separately at each imaging session and considered noteworthy if they fall outside of normal range and / or the subject is symptomatic: Subjects whose baseline ranges start outside of normal range will be assessed if they are symptomatic and / or meet criteria for assessment due to change in readings as noted in table below The following changes from baseline will be considered noteworthy:

Observation	Normal Range	Change for Assessment
Heart rate	50 – 110 beats/min	> 30 beats per minute
Systolic Blood Pressure	80-140 mm Hg	> 30 mm Hg
Diastolic Blood Pressure	60-90 mm Hg	> 20 mm Hg

Heart rate:	> 30 beats per minute
Systolic blood pressure	> 30 mm Hg
Diastolic blood pressure	> 20 mm Hg

Noteworthy changes will be documented on the Case Report Forms (CRF). The Principal Investigator will indicate on the CRF whether or not the changes in vital signs are clinically significant. If clinically significant, the principal investigator will assess the causality of the change to the injection of ^{18}F-FFNP or PET/CT imaging. Clinically significant changes in vital signs will be followed as needed until they return to baseline or normal levels, or until follow-up is no longer warranted. If a clinically significant change of a vital sign is noted, it will be reported on the adverse event log.

6.5 Image Analysis

The emission images will be corrected for measured attenuation using CT data according to the provide scanner manufacturer software package. FDG-PET/CT images will be evaluated and reported according to standard of care imaging procedures.

FFNP-PET/CT images will be evaluated by one observer qualitatively. PET images also will be evaluated semiquantitatively with the knowledge of the location of the lesion(s) by the use of the standardized uptake value (SUV) and tumor-to-normal tissue (T/N) ratio. The SUV is widely used for assessment of regional tracer accumulation in oncological studies, is technically simple to perform, and makes imaging easier for the patient because longer dynamic imaging is not required. SUV is a decay-corrected measurement of activity per volume of tissue (nCi/mL) divided by the average activity per unit mass in the entire body. The absolute change and the percent change in uptake of ^{18}F-FFNP will be assessed semiquantitatively and correlated with the clinical and radiologic results and subsequently with the results of the clinical follow-up evaluation. In patients with multiple lesions, the uptake up to 5 most intense lesions seen on PET images will be determined and the overall average values for all of the known lesions also will be recorded. The changes in SUV and T/N between the baseline and post estradiol challenge FFNP-PET/CT images will be compared in responders and nonresponders. Volumes of interest (VOIs) will be drawn around the entire lesion with the knowledge of the location of the tumor. SUVmax will be determined within the VOI. In addition, a similar volume of interest will be drawn in a comparable normal tissue region. The T/N ratio will be calculated by dividing the SUV_{max} of the tumor by the average SUV of normal comparable tissue. The absolute change and the percent change in uptake of ^{18}F-FFNP will be assessed semi-quantitatively and correlated with the clinical and radiologic results and subsequently with the results of the clinical follow-up evaluation. In patients with multiple lesions, the uptake of up to 5 lesions, selected as the most intense lesions seen on PET, will be determined and the overall average values for all of the known lesions also will be recorded. In addition, in patients with multiple lesions, SUV_{max} and T/N ratio will be measured for all known measurable lesions to assess within-patient heterogeneity in ^{18}F-FFNP uptake. Considering the optimum cutoff value for ^{18}F-FFNP uptake that distinguishes responders from nonresponders, lesions will be classified as ^{18}F-FFNP+ for lesions with ^{18}F-FFNP uptake at or greater than the cutoff value and ^{18}F-FFNP– for lesions with ^{18}F-FFNP uptake below the cutoff value. The changes in SUV and T/N after treatment will be compared in responders and nonresponders. The results of the PET studies will not be provided to the patient or the treating oncologist (see above). Clinical follow-up will provide information on tumor response, which will then be correlated with the sequential PET results to determine if these are predictive of ultimate response to estrogen therapy.

6.6 Toxicities Related to ^{18}F-FDG &-^{18}F-FFNP PET/CT Imaging

Likely:

- Mild discomfort from the placement of the IV in the patient's arm.

Less Likely:

- Discomfort from lying still on the imaging table.
- There is a slight risk of bruising at sites of vein puncture.

Rare:

- There is a remote risk of infection and an even smaller risk of blood clot at the site of the IV placement
- There is a rare possibility of an allergic-type or other adverse reaction to radioactively labeled drugs. While none have been reported to date with the

radioactive materials $^{18-}$F-FFNP or ^{18}F-FDG, such a reaction could be serious and may result in death.

- RADIATION EXPOSURE FROM PET/CT IMAGING: the amount of radiation exposure the patient will receive from one injection (15mCi) of ^{18}F-FDG and the CT scan for attenuation correction is equivalent to a uniform whole body exposure of approximately 1.51 rem. The amount of radiation exposure from ^{18}F-FFNP injection and the CT scan for attenuation correction is equivalent to a uniform whole body exposure of approximately 1.39 rem. Patients will be scanned on 2 separate occasions with FFNP plus one FDG scan resulting in a total exposure of 4.28 rem.

6.7 Toxicities Related to Estradiol Challenge

Because of the low dose and single administration toxicities from the estradiol challenge are not expted. The following side effects have been reported by patients taking estradiol as treatment for breast cancer over an extended period of time:

Likely/Common

- upset stomach
- nausea
- vomiting
- changes in appetite
- generalized feeling of weakness or fatigue
- fever
- headache

Less Likely/Less Common

- weight gain
- fluid retention
- insomnia (unable to sleep) or drowsiness
- vaginal discharge
- spotting to darkening of the skin
- breast discomfort or enlargement

Rare

- uterine fibroids
- stroke
- blood clots
- allergic reaction and symptoms such as-unexplained rash, itching, hives, and swelling, irregular heartbeat, difficulty breathing and shortness of breath.
- There is a rare possibility that the administration of estrace will cause symptoms of clinical flare. Clinical flare can occur with any hormone used in the treatment of breast cancer. Physicians recognize that women who experience a

clinical flare are likely to benefit from hormone therapy. Clinical flare is a temporary worsening of the symptoms associated with your breast cancer such as increased bone and joint pain.

6.8 Evaluation of Tumor Response

The patients will be followed by their treating oncologist every 3 months per standard of care scheduling and not dictated by protocol (or earlier in case of a suspicion of early progression) until disease progression. Clinical benefit (compete response + partial response + stable disease) will be determined ≥ 6 months after initiation of therapy. Response will be evaluated according to RECIST 1.1 (Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). Eur J Cancer 2009; 45:228-47). This evaluation will be based on the composite results of imaging studies (CT, MRI, bone scintigraphy, FDG-PET, etc.), serum tumor markers and evaluation of symptoms as deemed appropriate by the treating physician at the 6-month visit or at the time that there is evidence of clinical progression of disease, if before six months. Clinical response using RECIST version 1.1 by an oncologist will be defined as:

6.7.1 Complete Response:
Disappearance of all target lesions: Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

6.7.2 Partial Response (PR):
At least a 30% decrease in the sum of the longest diameter (LD) of target lesions taking as reference the baseline sum LD.

6.7.3 Progression (PD):
At least a 20% increase in the sum of the LD of target lesions taking as references the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

6.7.4 Stable Disease (SD):
Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started. Patients having a documented response with no reconfirmation of the response will be listed with stable disease.

6.7.5 Osseous disease only or osseous-dominant disease:
For patients with osseous metastasis only, a complete response was defined as disappearance of all objective and clinical disease, including complete normalization of radiological studies and tumor markers. A partial response was defined as a decrease in pain with evidence of recalcification of known osseous lesions on radiography. Disease progression was defined as worsening of disease on nuclear medicine scan or radiographs or worsening of pain and decline in

performance status. Any response that did not meet the criteria for complete response, partial response, or progression was defined as stable disease.

6.9 FOLLOW UP PROCEDURES

Follow up will consist of 2 parts. To assess for adverse events, approximately 24 ± 6 hours following injection, a follow-up telephone call will be made to the patient, or if the patient chooses another family member who will be able to assess for adverse events. In the event the patient is scheduled to be at the hospital for other testing or appointments, the assessment can also be made in person

Clinical follow-up via chart review will occur to document clinical progression of disease or change in therapy. Follow up chart review to assess for overall treatment response will also be conducted. A chart review will be conducted periodically until recurrence, progression or change in treatment. Data collected from the chart will include office notes from treating physicians, laboratory test results used to determine overall response and radiology reports and images. Response will be determined by the treating physician(s)

7.0 STUDY CALENDAR

	Screening	Baseline	Estradiol Challenge[3]	Post Estradiol	Follow-Up
Informed Consent	X				
Standard of Care chart review/Data collection[1]	X				X
Serum Estradiol level blood draw		X	X	X	
^{18}F-FFNP -PET/CT Imaging[2]		X	X	X	
^{18}F-FDG-PET/CT Imaging[4]	X				

1. Chart review/ data collection consists of obtaining source documents for eligibility check at screening and records pertaining to eligibility and treatment response as specified in section 6.6 at follow-up time point(s)
2. FFNP-PET/CT imaging one 2 separate occasions as described in section 6.0-6.2 of protocol and follow up for adverse events as described in section 6.7
3. Estradiol challenge 18F-FFNP-PET/CT imaging can be completed as soon as the following day as described in section 5.3. A maximum of 4 weeks is allowed between the baseline and estradiol challenge scans
4. If performed, FDG-PET/CT imaging must be done on day separate from FFNP-PET/CT. Preference is for FDG to be scanned prior to FFNP but alternative scheduling options may be necessary

7.1 Data Submission Schedule

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
Eligibility Form	Prior to baseline imaging session
PET Imaging Form	At time of each imaging session
PET Image Analysis	Approximately 24 weeks after estradiol FFNP-PET/CT challenge scan

8.0 REGULATORY AND REPORTING REQUIREMENTS

Subjects will be monitored for adverse events during the actual imaging period. Since FDG-PET/CT imaging is provided per standard of care no adverse event assessments will be recorded. For FFNP-PET/CT imagings, subjects will be contacted by phone or in person approximately

24± 6 hours after the injection of ^{18}FFNP to assess for adverse events as related to ^{18}F-FFNP injection, or PET/CT imaging. Any adverse events that occur within 24 hours of administration of ^{18}F-FFNP or within 24 hours of the first dose of estradiol which are graded related or possibly related to participation in the research will be reported according to the guidelines below:

8.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject who receives ^{18}F-FFNP-PET/CT and / or estradiol as part of the estradiol challenge for research purposes only, including any abnormal sign, symptom, or disease. The event does not necessarily have to be causally related to injection of ^{18}FFNP or PET/CT imaging to qualify as an adverse event, just temporally related.

Grading: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: (http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm).

8.2 Unanticipated Problems

Definition:

- Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.3 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

8.4 Serious Noncompliance

Definition: noncompliance that materially increases risks that result in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

8.5 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are *under the research team's control.*

Exceptions apply only to a single participant or a singular situation. Research imaging protocols which involve the injection of radioactive tracers can produce unique situations not common to standard treatment protocols. In the event a situation occurs which requires deviation from this protocol- for example less than expected tracer production, problems with the scanner, patient unable to tolerate the imaging protocol as described,- the principal investigator will have final authority over whether or not a study is completed. Any protocol deviations will be documented on the PET imaging data form. Deviations such as less than expected tracer production can be accounted for during data analysis and will not necessarily result in cancellation of the scan

Except as described above, pre-approval of protocol exceptions must be obtained prior to the event.

8.6 Reporting to the Human Research Protection Office (HRPO) and the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within 10 working days of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within 1 working day of the occurrence of the event or notification to the PI of the event.

8.7 Timeframe for Reporting Required Events

Deaths	
Any **reportable** death while on study or within 30 days of study	Immediately, within 24 hours, to PI and the IRB
Any **reportable** death while off study	Immediately, within 24 hours, to PI and the IRB
Adverse Events/Unanticipated Problems	
Any **reportable** adverse events as described in Sections 8.1 and 8.2 (other than death)	Immediately, within 24 hours to PI and within 10 working days to the IRB
All adverse events regardless of grade and attribution should be submitted cumulatively	Include in DSM report
Noncompliance and Serious Noncompliance	
All noncompliance and serious noncompliance as described in Sections 8.3 and 8.4	Immediately, within 24 hours, to PI and within 10 working days to the IRB

9.0 DATA AND SAFETY MONITORING PLAN

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date and
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules

- Summary of toxicities
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

10.0 STATISTICAL CONSIDERATIONS

10.1 Study Objectives and Endpoints

The primary endpoints of the study are the change in ^{18}F-FFNP uptake following one-day of estradiol measured as the SUV, T/M and tumor-to-normal tissue ratio (T/N), and the response to ET among cancer patients (responders vs. non-responders). The secondary endpoint of the study is the immunohistochemical (IHC) determination of PgR status (PgR+ vs. PgR–) in ER+ breast carcinoma.

Study Design: This is a pilot study aiming to evaluate if response to ET of breast cancer can be predicated by the changes in tumor uptake of ^{18}F-FFNP after a one-day estradiol challenge test. The sampling method is non-random. The change of ^{18}F-FFNP uptake after estradiol will be correlated with responsiveness to ET. Additionally, an optimal threshold of the change in ^{18}F-FFNP uptake will be developed to differentiate therapy responding and non-responding patients. Also, the change in ^{18}F-FFNP uptake after estradiol challenge will be compared with the IHC determination of PgR status with respect to the prediction of ET responsiveness.

Accrual: The rate of accrual for the study is expected to be about 1 - 2 patients per month. It is expected that the accrual period of the study will be completed in 30 months with total 50 patients enrolled.

Power Analysis: It is estimated that the response rate to ET in the recruited patients will range from 20-50%. Using a 2-sided independent t-test with 80% power at a 0.05 significance level, a sample of 10 responders vs. 40 non-responders (i.e., 20% response rate) will allow us to detect a minimum of 101% SD between-group difference, where SD represents a pooled standard deviation of the ^{18}F-FFNP uptake changes among both therapy responding and non-responding patients; and a sample of 25 responders vs. 25 non-responders (i.e., 50% response rate) will allow us to detect a minimum of 80.9% SD between-group difference.

Data Analysis: Demographic and clinical characteristics of all the enrolled patients will be summarized using descriptive statistics. The changes in ^{18}F-FFNP uptake after estradiol challenge will be compared between responders and non-responders via

Wilcoxon rank sum test. A receiver operating characteristic (ROC) curve will be plotted to identify an optimal threshold of ^{18}F-FFNP uptake change to determine a criterion for the future prediction of therapy responsiveness. The positive and negative predictive values (PPV and NPV) will be calculated for response to ET using both the ^{18}F-FFNP uptake-based criterion and IHC determination of PgR receptor status. Multivariate logistic analyses of therapy response will be used to examine prediction power among the changes in ^{18}F-FFNP uptake and PgR receptor status

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