6.0 Study Protocol

Local Protocol #: 7184

TITLE: Serial [F-18] fluoroestradiol (FES) PET Imaging to Evaluate Endocrine-Targeted Therapy

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6.1 SCHEMA

This clinical study will investigate the utility of [^{18}F]FES in patients with estrogen receptor (ER) positive, primary, recurrent or metastatic, breast carcinoma. [^{18}F]FES PET has been validated as a measure of estrogen receptor expression. This trial is also designed to test the safety of [^{18}F]FES. In addition, this study will obtain data on the value of [^{18}F]FES to predict and evaluate response to endocrine-targeted therapies as a measure of ER expression in this patient population. If promising, these data will be used to design a phase II multi-center clinical trial.

We anticipate that the imaging measure of receptor density will predict the likelihood that a patient will respond to hormone targeted therapy, evaluate subsequent changes in this measure as the patient undergoes therapy and that eventually [^{18}F]FES imaging will help us design therapeutic trials for better treatment outcomes. We also expect that repeat [^{18}F]FES PET imaging shortly after the start of endocrine-targeted therapy will reflect the pharmacodynamics of endocrine targeted therapy.

This protocol is outlined in the following schematic. Briefly, patients with primary, recurrent or metastatic breast cancer from an ER+ primary tumor who will be undergoing endocrine-targeted therapy will be imaged with [F-18]-16α-fluoroestradiol. All patients will undergo a pre-therapy FES PET or FES PET/CT scan (hereafter FES PET will be used to denote both FES PET and FES PET/CT unless specifically noted). In some patients receiving non-interfering medications such as aromatase inhibitors, scans may be performed with patients already on a treatment who are to start an alternative or additional endocrine targeted treatment. Patients will undergo a repeat FES PET and FDG PET on study after starting the endocrine-targeted therapy. The exact timing of the repeat scans may vary depending on the treatment regimen. In the repeat studies, we will be testing the ability of FES PET and FDG PET to measure the pharmacologic effect of endocrine-targeted therapy and early measures that may help predict response to such targeted agents. In patients on selective estrogen receptor degrader (SERD) therapies, where repeat FES PET is used primarily to evaluate residual ER availability rather than tumor response, repeat FDG PET may be omitted, as determined by requirements of the co-enrolling companion therapeutic protocol. A subset of patients may undergo a third FES PET at a later time point determined by the treatment regimen.

FDG PET and CT will be used as staging tests for this study and FDG will also serve as a comparator for tumor localization with the FES imaging. Patients will have a clinical FDG PET scan prior to the start of treatment as part of their standard care; their treating physician will order this. In rare cases where an FDG PET has recently been performed at an outside medical facility but the scan has (1) inadequate image quality or (2) insufficient quantitative information, or for patients who would not be ordered as a standard part of the patient's care, a baseline FDG scan will be done as part of this protocol. A second FDG PET scan will be done as a study procedure concurrent with the FES PET timing stipulated by treatment regimen (this may vary from 1 to 12 weeks post treatment start depending on regimen). In the subset of patients undergoing a third

FES PET scan there may also be a research FDG PET done at the same time point if determined by the treatment regimen.

After the FES and FDG baseline imaging (see schematic below), the patients will start or change therapy, typically within 30 days, and be followed clinically for up to 20 years. Lesion location will be identified using clinical FDG scans with reference to clinical CT for metastatic disease or ultrasound, mammography, and/or breast MRI for primary tumors in patients presenting with metastatic or recurrent disease that still have a primary breast lesion. FES and FDG images will be spatially co-registered and tumor regions will be drawn on the FDG images and transferred to the FES images for quantitative analysis of FES uptake in both ER positive and ER negative tumors. For FDG studies, SUVs will be generated.

The FES uptake from images will be analyzed qualitatively by visual assessment and quantitatively using a standard uptake value (SUV). Serial measurements of hormone levels in peripheral blood may be used with some treatment regimens to allow determination of efficacy of endocrine therapy. ER, PR and HER2 assays will allow determination of hormone receptor status and whether HER2 over-expression indicates estrogen-independent growth in some treatment studies, however, there may not be pathologic correlates in all studies.

We emphasize that this is an observational study in that [^{18}F]FES PET will not be used to direct therapy. Treatment regimens are chosen by the referring oncologist prior to the [^{18}F]FES PET study on the basis of clinical criteria or participation in experimental treatment protocols. While patients and referring physicians will not be blinded to [^{18}F]FES PET results, treatment choices will be made prior to the entry of the patient into the study and will not be altered because of the FES PET results.

Patients with metastatic or recurrent disease may consent to optional research biopsies performed prior to start of the selected endocrine-targeted therapy, and after 1-12 weeks on therapy. Obtained tissues will be analyzed for expression of prognostic markers and/or for intra-tumor steroid hormone concentrations to evaluate correlations between results of imaging and histo-pathological studies and to enhance understanding of mechanisms of response and resistance in the context of tumor microenvironment.

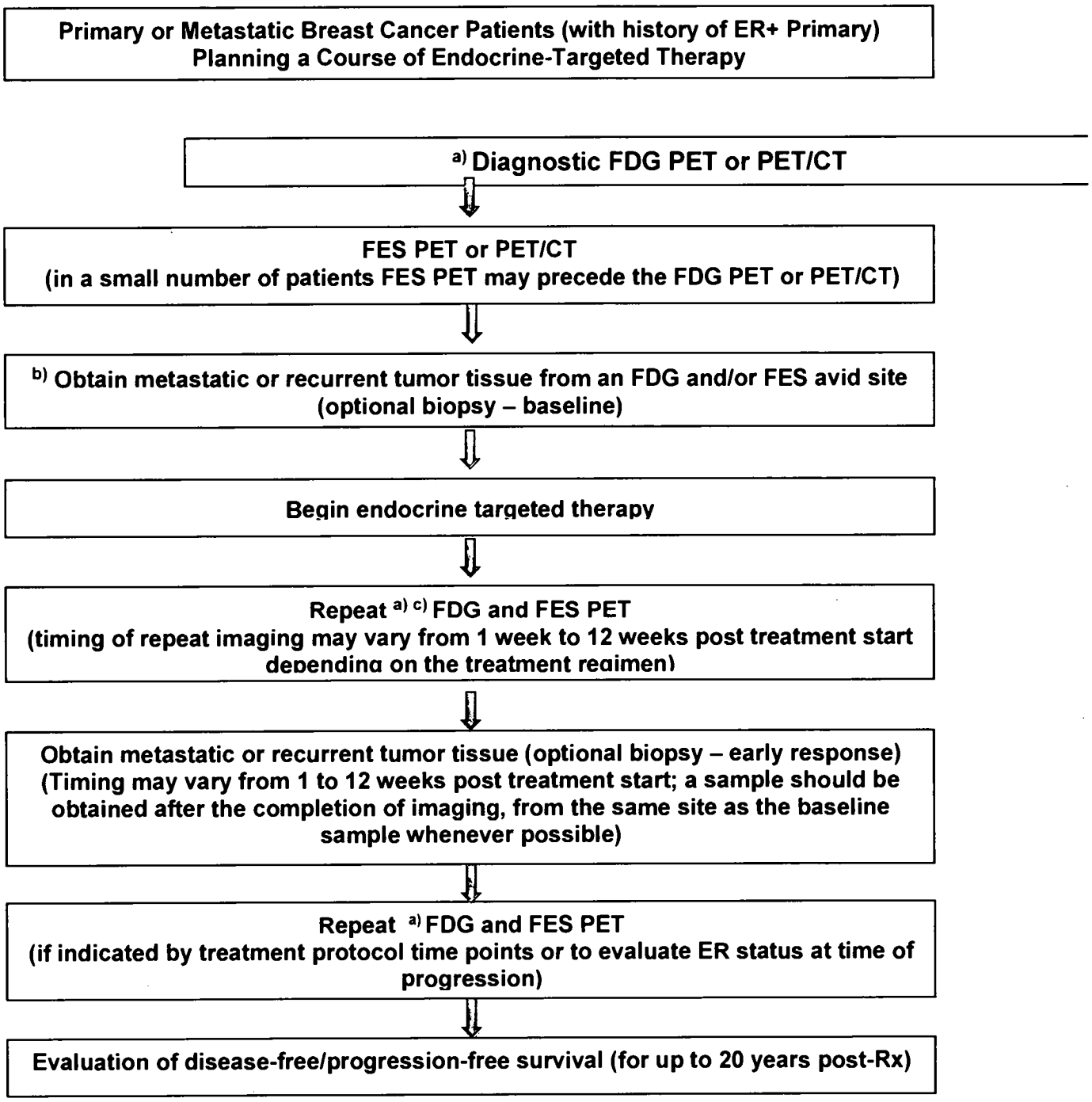

Figure 6.1 Outline (Schema) of the [^{18}F]FES Study Protocol. [a)] FDG PET is not required for primary breast cancer patients unless required for localization or requested by treating physician. [b)] Where available and medically appropriate, a clinical or research biopsy that involved sampling of a relevant lesion within 6 months prior to the enrollment may be utilized in place of the baseline optional biopsy. [c)] Repeat FDG PET may be omitted in patients on SERD therapies.

6. 2 OBJECTIVES

6.2.1 Primary Objective

Measure the effect of endocrine targeted therapy on ER expression and estradiol binding to the receptor using serial FES PET and FDG PET.

6.2.2 Secondary Objectives

Document the safety profile of FES PET in patients with breast cancer.

Examine associations between FES PET results and serial measurements of hormone or other levels in peripheral blood, as related to efficacy of endocrine-targeted therapy. Correlate FES PET uptake measures with histopathological assays and tumor microenvironment studies on biopsy specimens, if relevant to specific treatment regimen.

6.3 BACKGROUND

6.3.1 Study Disease

6.3.1.a Invasive breast carcinoma: In 2007, breast cancer will be diagnosed in over 180,000 women and account for over 40,000 deaths in the United States (Jemal, 2007b). The incidence of hormone receptor positive disease appears to be increasing (Li, 2003). Advances in diagnosis and therapy have resulted in increased survival for early breast cancer (Jemal, 2007a; Piccart-Gebbhart, 2005; Romond, 2005; Winer, 2005; Jemal, 2006). This increase in breast cancer survival may result in more patients diagnosed with advanced or metastatic breast cancer. Improved and targeted treatment of advanced breast cancer has also resulted in improved outcomes (Chia, 2003). However, better tailoring of therapy to patients is likely to result in higher quality of life (Greenberg, 1996).

6.3.1.b Invasive breast carcinoma and hormone receptors: Nearly two-thirds of breast cancer patients have hormone receptor bearing (HR+) tumors (Carlson, 2002). Treatment options for HR+ patients have expanded to more potent and less toxic therapies, inviting clinicians to offer endocrine therapy to more patients (Carlson, 2002; Lonning, 2001). Nonetheless, chemotherapy is frequently offered to patients without proof of superior outcomes. Measurement of tumor hormone receptor expression, both estrogen receptor (ER) and progesterone receptor (PgR) at the time of primary diagnosis is now the standard of clinical care (Carlson, 2004). Knowledge of hormone receptor expression is essential for selection of

appropriate therapy but biopsies and serum assays may not provide sufficiently complete information (Anonymous, 1998; Osborne, 1998).

6.3.1.c Treatment of breast carcinoma: Treatment selection for breast cancer poses several challenges. Heterogeneity is a challenge in management of breast cancer, especially for locally advanced or metastatic disease. In cases where tissue sampling is feasible, the expression of ER may be heterogeneous, and ER expression at one site does not guarantee expression at all sites. This issue presents significant practical challenges for assessing ER expression in breast cancer. The potential for misidentification of patients appropriate for endocrine therapy is significant. While ER expression predicts measurable tumor regression (objective response) in 30% to 77% of patients with a new diagnosis of breast cancer, response rates are more typically 7% to 21% in patients with recurrent disease and prior breast cancer treatment (Lonning, 2001). A predictive assay of hormone sensitivity capable of assessing ER expression at all sites of disease would be highly valuable for selection of which patients are more likely to benefit from endocrine therapy.

6.3.1.d Recent advances in endocrine therapy: A significant advance in the management of ER+ metastatic breast cancer is the introduction of aromatase inhibitors (AI) (Winer, 2005; Mouridsen, 2001; Nabholz, 2000). These agents are more effective and result in a longer time to progression than previous therapies, and have therefore displaced tamoxifen as first-line treatment for metastases and adjuvant therapy (Winer, 2005; Mouridsen, 2001; Nabholz, 2000, Carlson 2005). However, several endocrine therapies are active agents for the treatment of hormone responsive disease: fulvestrant, a selective estrogen receptor demodulator agent or SERD; tamoxifen, a selective ER blocking drug, or SERM; as well as megestrol, testosterone, and diethylstilbesterol (DES) (Lonning, 2001; Carlson, 2005; Osborne, 2002; Buckner, 1989). Increasing use of AIs in the adjuvant setting will lead to increased use of alternative endocrine treatment strategies in the metastatic setting.

6.3.1.e Endocrine therapy for breast carcinoma: The optimal management of patients who experience progression of disease while on adjuvant aromatase inhibitor treatment, and of premenopausal patients, remains under investigation. There is evidence to support the usefulness of irreversible steroidal AIs (exemestane) for treating patients who progress on nonsteroidal AIs (Lonning, 2000). Clinical experience suggests a primary role for this strategy in patients who have a significant disease-free interval on a hormonal adjuvant therapy. Currently active investigational protocols will determine the optimal management of premenopausal women with hormonal therapy. However, tamoxifen remains standard management and tamoxifen therapy in pre-menopausal patients may be complicated by high estradiol levels due to tamoxifen

stimulation of the ovary (Jordan, 1991; Sunderland, 1991). Ovarian suppression alone or with tamoxifen or an AI is also an effective strategy (Carlson, 2004; Davidson, 2000; Klijn, 2000; Klijn, 2001; Milla Santos, 2002), but the optimal sequence of endocrine treatment in premenopausal patients is not yet clear (Prowell, 2004). Our study will test the value of FES PET for predicting the effectiveness of endocrine therapy, with the eventual goal of selecting patients with primary or metastatic disease most likely to benefit from this expanding choice of agents for endocrine therapy. In addition, our hypothesis is that FES PET will be useful as a predictive assay for those patients who have failed one endocrine therapy agent and are being considered for an alternative endocrine treatment.

6.3.1.f Imaging of ER with [^{18}F]FES: PET ER imaging using [^{18}F]-fluoroestradiol (FES) poses an attractive possibility to meet this clinical need. Previous work evaluating the ER binding, radiation dosimetry, blood clearance and protein interactions of FES in women with hormone receptor positive breast cancer has shown that uptake correlates with in vitro assay of expression in breast cancer tissue samples (Mankoff, 2001; Mintun, 1988) . Furthermore FES positron emission tomography (with either PET or PET/CT) measures the heterogeneity of ER expression and function in metastatic disease (Mankoff, 2002; Dehdashti, 1995). Prior studies have shown that the level of FES uptake predicts the response of advanced breast cancer to hormonal therapy; these therapies were predominantly tamoxifen (Mortimer, 2001). We also showed that the level of FES uptake as an in vivo indicator of ER function predicted response to endocrine therapy (Linden, 2006). We measured FES uptake in patients with recurrent or metastatic breast cancer treated largely with aromatase inhibitors. In this study we will test FES predictive capability in patients undergoing endocrine therapy of breast cancer, and also to test the effect of ER-directed breast cancer treatments on ER expression and/or ER blockade.

6.3.2 Investigational Agent

6.3.2.a 16α-[^{18}F]-fluoro-17β-estradiol (FES) is a radiolabeled imaging agent that has been used for investigating tumor estrogen receptor activity with positron emission tomography (PET). It was developed in a collaboration of the Welch and Katzenellenbogen laboratories (Kiesewetter, 1984) as a positron-emitting radiopharmaceutical for imaging ER expression. Studies have shown that the strength (K_D) of FES binding to the ER is nearly identical to estradiol (Kiesewetter, 1984). FES binding to sex steroid transport protein (SBP or SHBG) is also nearly identical to estradiol (Kiesewetter, 1984; Tewson, 1999). Fluorine-18 labeled FES can be synthesized with high specific activity so the quantity of estrogenic material injected with the radiopharmaceutical is <5 μg (Kiesewetter,

1984; Lim, 1996). The typical peak molar concentrations of [^{19}F]FES from a [^{18}F]FES injection is 1 pmol/mL, a value comparable to circulating estrogen levels. This value decreases by 60 minutes post-injection to 0.05 pmol/mL. [^{18}F]FES can generate high quality PET images of regional estradiol binding using a 6 mCi injection, with acceptable radiation dose to the patient (Mankoff, 2001). In breast cancer, the uptake of FES measured by PET has been shown to correlate with ER expression in biopsy material assayed by in vitro radioligand binding (Mintun, 1988) or by immunohistochemistry (Peterson, 2008). As has been found for in vitro assays of ER expression, the level of FES uptake in breast cancer is predictive of response to endocrine therapy in advanced tumors treated with primary tamoxifen therapy (Mortimer, 2001) and metastatic cancer treated by salvage hormonal therapy, mostly in the form of aromatase inhibitors (Linden, 2006). This protocol will evaluate FES as an in vivo predictive assay for endocrine therapy of newly diagnosed metastatic breast cancer.

6.3.3 Rationale

6.3.3.a The primary hypothesis to be tested is that pre-therapy and post-therapy [^{18}F]FES uptake will measure the effect of endocrine-targeted therapy on ER expression and/or estradiol binding to the ER.

6.3.3.b This single-site study will determine the ability of [^{18}F]FES to quantify changes in ER expression and estradiol binding in vivo in patients with breast cancer for whom endocrine-targeted therapy has been chosen based on clinical presentation or as part of therapeutic clinical trial. The results of this trial will help evaluate the effect of ER-directed therapies on ER expression and estrogen binding to ER, and secondarily whether the FES PET measures of estrogen receptor function, and changes with treatment, predict response to endocrine targeted therapy in patients with known ER+ primary or metastatic breast cancer as measured by immunohistochemistry (IHC) of biopsy material. The primary goal is to gain insight into the efficacy of ER-targeted therapies, especially novel therapies, on ER expression and estrogen binding to ER. For example, serial FES PET imaging could be used to test the efficacy of an ER antagonist in blocking estradiol binding, or the ability of an agent directed at altering ER expression to change ER expression levels in tumors. Such in vivo measures of the pharmacodynamics of ER-directed therapy have traditionally required serial biopsy and have been quite challenging. FES PET presents a non-invasive alternative to measure the effects of ER-directed therapy.

6.3.3.c We have examined the role of FES in 47 heavily pretreated patients (Linden, 2006). Initial FES uptake was measured and correlated with subsequent tumor response to 6 months of hormonal treatment. In this

study, 11/47 (23%) patients had an objective response. Quantitative FES uptake and response were significantly associated; 0/15 patients with initial FES SUV<1.5 responded to hormonal therapy, compared to 11/32 (34%) with FES SUV ≥1.5 ($p < .01$). In the subset of patients whose tumors did not over-express HER2/neu, 11/24 (46%) of patients with SUV ≥ 1.5 responded. These pilot results support the further evaluation of the predictive capability of FES PET.

6.3.3.d FES uptake predicted a greater likelihood of response to endocrine therapy. Patients with low or absent FES uptake were unlikely to manifest a response to treatment, yet FES did not help to distinguish which patients would experience a clinical benefit, defined as stable disease. Several factors may have contributed to this finding. Stable disease over 6 months included patients with disease that may have progressed slowly, independent of treatment, as well as patients with rapidly progressive disease in whom therapy slowed but did not abrogate tumor growth. The study was not designed to distinguish between these two outcomes. In addition, we identified PgR (progesterone receptor) expression as contributing to likelihood of response, and noted that elevated estradiol levels and HER2 expression were associated with non-response. In this new study we propose to document tumor characteristics including quantitative ER mRNA levels measured by PCR and semi-quantitative levels of ER, PgR, AR and HER2 by IHC . Gathering these indices as part of the proposed study may help to enhance the predictive value of PET FES, allow understanding of mechanism of resistance to endocrine therapy, and potentially determine which patients are likely to appreciate clinical benefit as a result of endocrine therapy, even in the absence of an objective response.

6.3.3.e In prior observational imaging trials (unpublished data) we have measured regional estrogen-ER binding using FES PET before and after treatment with estrogen depleting therapies, Aromatase inhibitors (AI), and estrogen blocking therapies, Tamoxifen (TAM), or fulvestrant (FUL) in 30 metastatic breast cancer patients. As expected, tumor FES uptake declined markedly on ER blockers TAM and FUL (average 54% decline), but less than 15% on average on estrogen-depleting AIs ($p<0.001$). All 5/5 patients taking TAM showed complete tumor blockade (FES SUV ≤ 1.5 after treatment), compared to 4/11 of FUL patients ($p=0.009$). We concluded that molecular imaging can assess the *in vivo* pharmacodynamics of targeted agents, and may give insight into the activity of established therapeutic agents.

6.4 PATIENT SELECTION

6.4.1 Inclusion Criteria:

6.4.1.a Adult, non-pregnant patients with biopsy-proven or clinically obvious primary, recurrent or metastatic breast cancer

6.4.1.b Breast cancer from ER+ primary that is seen on other imaging tests. Tumor ER expression must have been confirmed by immunohistocytochemistry of primary tumor or recurrent disease.

6.4.1.c At least one site of disease 1.5 cm or greater is needed to meet the spatial resolution limits of PET imaging.

6.4.1.d Patients must have been off tamoxifen or other estrogen receptor blocking agents for at least 6 weeks and off chemotherapy for 3 weeks for the initial baseline FES.

6.4.1.e Patients must be selected for an endocrine targeted therapy regimen for treatment of their breast cancer by the referring oncologist. Selected treatments may be part of experimental treatment protocols for which the patient would be separately consented.

6.4.1.f Patients must be willing to undergo serial imaging procedures.

6.4.1.g Patients must agree to allow access to clinical records regarding response to treatment and long term follow up.

6.4.2 Exclusion Criteria:

6.4.2.a An inability to lie still for the tests

6.4.2.b Individuals weighing more than 300 lb. (this is the weight limit of the scanner table)

6.4.2.c Pregnant or lactating. Women of childbearing potential with either a positive or no pregnancy test at baseline are excluded.

6.4.2.d Any other life-threatening illness (e.g. serious, uncontrolled concurrent infection or clinically significant cardiac disease – congestive heart failure, symptomatic coronary artery disease, cardiac arrhythmia not well controlled with medication).

6.4.2.e Use of tamoxifen, faslodex, DES or any other ER blocking agent < 6 weeks or chemotherapy < 3 weeks prior to imaging scan.

6.4.2.f Unwillingness or inability to give informed consent.

6.4.2.g Uncontrolled diabetes mellitus (fasting glucose > 200 mg/dL)

6.4.2.h Adult patients who require monitored anesthesia for PET scanning.

6.4.3 Inclusion of Women and Minorities:

Refer to Section 6.14.4

6.5 REGISTRATION PROCEDURES

Not applicable; this is a single-institution trial

6.6 TREATMENT PLAN

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 6.8.1. There will be no dose modifications for FES administration. No investigational or commercial agents or therapies other than those described below will be administered with the intent to treat the patient's malignancy.

6.6.1 [^{18}F]FES Administration:

FES will be administered in the PET imaging suite at the University of Washington Medical Center or at the Seattle Cancer Care Alliance (SCCA). The [^{18}F]FES for injection will be prepared as described in section 6.6.2, Study Procedures and Schedule of Events. Upon arrival, the patient will have an opportunity to have questions answered regarding the procedure. The patient will have an intravenous line placed prior to [^{18}F]FES administration. A dose of nominally 220 MBq (6 mCi with a range of 110 to 220 MBq = 3 to 6 mCi) of [^{18}F]FES will be administered intravenously by a physician. The injection will be infused over approximately 2 minutes and followed by a saline flush. The infusion and imaging procedure will be terminated in any patient who exhibits anaphylaxis, physical signs of significant hypotension, significant dyspnea or chest pain. We do not expect to observe any of these effects as no events have been reported in more than 300 scans performed with this compound at our institution to this date. The patient will then be allowed to rest comfortably in a prep room prior to being positioned in the PET scanner to commence the image acquisition at 60 +/- 10 minutes post FES injection.

Heart rate and blood pressure will be recorded pre- and post-FES dosing, and will continue to be monitored after injection until baseline is achieved.

Patients will be monitored for adverse events, potentially related to the administration of [^{18}F]FES, from the time of the initial injection to 10 hours post injection. Any adverse events will be recorded and reported according to FDA requirements (see section 6.8 Adverse Events).

6.6.2 Study Procedures and Schedule of Events

6.6.2.a Initial visits prior to [^{18}F]FES PET: Patients who are identified as potential candidates for the [^{18}F]FES PET study will be approached to explain the nature of the study and to obtain their written informed consent to be enrolled in the protocol. Heart rate and blood pressure will be recorded at the time of clinic visit where study is considered or at the time of consent. The patient will have a clinical FDG PET/CT scan if a recent FDG scan has not already been obtained. The FDG PET/CT scan should be obtained close to the time of FES PET/CT, typically within 14 days of the [^{18}F]FES PET/CT scan. An additional FDG PET/CT scan may be done as a research scan if the patient has undergone a recent PET scan at an outside institution or if they are unable to obtain a clinical PET scan as part of their clinical care. The research FDG PET/CT scan in this instance will be identical in procedure to a clinical FDG scan. The following additional patient data will be obtained: histological diagnosis, age at radiologic diagnosis, weight, gender, ECOG score and other treatment modalities used. Correlative radiology including CT, MRI, or bone scan, and laboratory tests measuring renal, blood and liver function will also be collected, typically for values within 30 days of the FES PET/CT scan. For patients referred from outside the UW health system, patient records, and biopsy material and imaging as necessary, will be reviewed to determine eligibility for the study.

6.6.2.b Day of [^{18}F]FES PET/CT Scan: After the FES injection and rest period (section 6.6.1), the patient will be positioned supine in the PET/CT scanner. A low dose CT scan (60 mA) will provide attenuation correction data using non-contrast CT. The PET image acquisition sequence will be the same as that used for clinical FDG scans. This consists of a torso survey series of five-minute images acquired over 15 cm survey fields of view (FOVs) extending from the base of the skull to the mid thigh (5 to 7 FOVs in total). The images will be corrected for radioactive decay of the tracer and normalized to the injected dose and body weight. This results in regional standardized uptake values (SUVs) whole-body image sets.

Prior to injection, a whole blood sample may be taken for determination of albumin, estradiol and testosterone levels, heart rate and blood pressure

will be recorded. A known quantity of nonradioactive FES will be added to plasma (from blood collected prior to injection) to determine binding to sex steroid binding protein (SBP). For patients in whom a second venous access line is feasible and has been placed, venous blood samples may be collected at 5, 20 and 60 minutes after injection of the [^{18}F]FES for metabolite analysis in selected patients. Heart rate and blood pressure will be measured after the FES injection.

6.6.2.c After the [^{18}F]FES PET Scan: A follow up phone call will be made by study staff within 24 hours after the FES PET scan to ask about any signs of adverse effects. Patients will typically start the selected hormone targeted therapy within 30 days of the FES PET scan. Patients who have started aromatase inhibitors prior to [^{18}F]FES PET will be permitted to enter the study, if an additional endocrine targeted treatment is planned.

6.6.2.d In patients who consent to optional biopsies, a baseline tumor sample will be obtained after completion of the baseline PET imaging but prior to starting the selected endocrine targeted therapy. Where available and medically appropriate, a clinical or research biopsy that involved sampling of a relevant lesion within 6 months prior to the enrollment may be utilized in place of the baseline optional biopsy.

In some patients undergoing biopsies, a whole blood sample will be collected prior to starting the therapy, to allow correlation of steroid hormone levels in serum and biopsy tissue.

6.6.2.e Repeat FES PET scan and FDG PET scan : Patients will undergo a second FES PET and FDG PET scan after starting endocrine targeted therapy to assess the effect of therapy on ER expression and estradiol binding to the receptor. Exact timing of the second FES and FDG scans, will depend upon the endocrine targeted regimen and dosing, but typically will be 1 – 12 weeks after starting therapy.
The repeat FDG PET may be omitted in patients on SERD therapies, where repeat FES PET is used primarily to evaluate residual ER availability rather than tumor response. This will be determined by requirements of the co-enrolling companion therapeutic protocol.

6.6.2.f In patients who consent to optional biopsies, an early response tumor sample will be obtained 1-12 weeks after starting the selected therapy. This biopsy should take place after completion of the repeat PET imaging and should target the same tumor site as the baseline biopsy. A whole blood sample may be drawn for correlation of steroid hormone levels in serum and biopsy tissue.

6.6.2.g Week 1 to 20 years after [^{18}F]FES PET Scan: Clinical follow-up will continue for up to 20 years, time of progression and date of death will be

recorded. For some patients, especially those receiving two endocrine-directed treatments, a third FES PET scan and additional research FDG PET scan may also be obtained 1-12 weeks after the second FES PET scan. For each patient, a maximum of 3 FES PET and 3 FDG PET scans would be done.

6.6.3 General Concomitant Medication and Supportive Care Guidelines

Because there is unlikely to be interaction of < 5 μg of [^{18}F]FES with other concomitantly administered drugs through the cytochrome P450 system, this section is not applicable.

The infusion and imaging procedure will be terminated in any patient who exhibits adverse reactions as described in section 6.6.1 and 6.8.1.

6.6.4 Duration of Therapy

As this is an observational study, the results of [^{18}F]FES PETs and repeat FDG PETs are not used to direct therapy, and the duration of therapy is determined on the basis of clinical grounds by the referring physician, and is not influenced by the FES study.

6.6.5 Duration of Follow Up

Patients will be followed up to 20 years after [^{18}F]FES PET. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

6.7 DOSING DELAYS/DOSE MODIFICATIONS

As this is a trial of a diagnostic imaging agent to be administered before endocrine therapy, dosing delays and dose modifications are not relevant to this protocol.

6.8 ADVERSE EVENTS: LIST AND REPORTING GUIDELINE

6.8.1 Adverse Events and Potential Risks

No adverse events have been reported for diagnostic [^{18}F]FES administration at the strength described for this study in 267 patients studied at the University of Washington. Thus no adverse effects are expected as a result of the administration of [^{18}F]FES. The [^{18}F]FES dose is less than 0.002 of the recommended safe oral dose assuming an oral conjugated

estrogen dose of 2.5 mg in one day. Unexpected but potential adverse effects are listed below.

Anaphylaxis, Hypotension, Dyspnea, Chest pain, Flushing, Euphoria
Infection at the injection site or systemic infection
Extravasation of the dose

6.8.2 Adverse Events Characterization

6.8.2.1 the general definitions for adverse events are:

Adverse Event: Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite). Each AE is a unique representation of a specific event used for medical documentation and scientific analysis.

Serious Adverse Event (SAE): An SAE is defined in the FDA CFR 312 as any adverse drug experience occurring at any dose that results in any of the following outcomes: death, a life-threatening adverse drug experience, inpatient hospitalization, or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. We will report serious AEs via expedited reporting to FDA and to our local IRB.

Life-Threatening Adverse Event: Any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death. AEs can be 'Unexpected' or 'Expected' for expedited reporting purposes only. A list of adverse events that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in Section 6.8.1.

In addition to oversight by the PIs, this study will be monitored by the UW/FHCRC Cancer Consortium Institutional Review Board and the Radiation Safety Committee.

6.8.3 Expedited Adverse Event Reporting

Expedited AE reporting for this study will be done through the Cancer Consortium IRB and FDA and as required by FDA MedWatch. These requirements are briefly outlined in the table below.

All life threatening adverse reactions reports are submitted to the FDA, our local IRB and to all investigators. A copy of the report is kept on file.

Table 6.iii.2 Reporting Requirements

	Unexpected			**Expected**
	Adverse Reaction (known or suspected attributable to the use of [^{18}F]FES)		**AE not attributable to [^{18}F]FES**	**AE, AR**
	Serious including life-threatening (or death)	Nonserious	Life-Threatening or serious or not serious	Not applicable to [^{18}F]FES
Reporting Time Requirement to the FDA	**Report to FDA ASAP and within 7 days of discovery of event (section 6.iii.8.7)**	Annual Continuation Review submission	Annual Continuation Review submission	Not applicable to [^{18}F]FES
Reporting Form for the FDA	IND Safety report of potentially serious risk	Annual Reports / Case reports	Annual Reports / Case reports	Not applicable to [^{18}F]FES
Reporting Time Requirement to the local IRB	**Report to IRB ASAP within 10 days of discovery of event** (suspected is defined as 50% probability attributable to [^{18}F]FES study) this also includes any increased risks with the study even without an AE	At continuation review time	At continuation review time	Not applicable to [^{18}F]FES
Reporing form for the IRB	Expedited Reporting Form for Unanticipated Problems or Noncompliance and Adverse Event Reporting Form	Form for Unanticipated Problems or Noncompliance, Case reports on continuation form, Data Saety Monoring Reports	Form for Unanticipated Problems or Noncompliance, Case reports on continuation form, Data Safety Monitoring Reports	Not applicable to [^{18}F]FES

6.8.3.a Expedited Reporting Guidelines

All deaths for subjects while on study will be reported to FDA using expedited reporting regardless of causality. Attribution to treatment or other cause will be provided.

Fatal and life-threatening events will be reported to the University of Washington IRB within 24 hours of notification of the event, indicating that a full report will follow. All adverse events will be submitted to the IRB within10 days of notification of the event.

Fatal and life-threatening events will also be reported within 24 hours to the FDA following the MedWatch guidelines set by the FDA in compliance with the 21 CFR 312.32.

Life-threatening (or fatal) adverse reactions must be reported within 7 days to the FDA. The FDA should be notified as soon as the adverse reaction is discovered by telephone or fax or email. The instructions and forms are available at http://www.fda.gov/Safety/MedWatch/HowToReport/default.htm The report should be sent ASAP by mail and followed with a follow-up report. Individual IND safety reports to FDA are submitted on the Medwatch FDA Form 3500A as an "IND Safety Report". The form should be sent to The Director, Office of Generic Drugs in the Center for Drug Evaluation and Research at FDA. The address and phone numbers are available at: http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm119100.htm.

6.9 PHARMACEUTICAL INFORMATION

6.9.1 Production of the Radiopharmaceutical

[^{18}F]FES will be produced at the University of Washington according to the methods described in Section 7 of this IND application, Chemistry, Manufacturing and Controls.

6.9.2 Reported Adverse Events and Potential Risks:

A list of the adverse events and potential risks associated with [^{18}F]FES was provided in Section 6.8.1.

6.9.3 Agent Accountability:

The chemical precursor for [^{18}F]FES is provided in house or by ABX in single-use, 2 mg vials that are delivered to the custody of Dr. Steven Shoner, the lead radiochemist for this project. They are stored in a controlled temperature freezer in a locked and secure room and they are

inventoried with a chain of custody maintained from the time of receipt. Each radiosynthesis is done by Dr. Shoner or his designee and, after passing all required quality control assays, the product [^{18}F]FES dose is drawn up under the supervision of the nuclear medicine physician investigators, Jean Lee, MD, Andrew Shields, MD or David Lewis, MD. The quality control tests that must be passed prior to release of the product [^{18}F]FES for injection include the pH, radioactive purity, radiochemical purity, specific activity, sterilizing filter integrity, tests for Kryptofix [2.2.2], acetonitrile, endotoxins and particulates. Acceptance criteria are detailed in the CMC document. The [^{18}F]FES dose is drawn up into a syringe, assayed for mCi at time of injection, labeled and administered to the research subject.

6.10 CORRELATIVE/SPECIAL STUDIES

6.10.1 FDG-PET Imaging

In most cases, a standard clinical diagnostic FDG PET or PET/CT scan will be obtained prior to the [^{18}F]FES PET scan. The FDG PET or PET/CT scan serves to help identify active sites of breast cancer and is used to help determine sites on the FES PET scan where estradiol binding would be expected. At least one site of measurable disease outside of the liver, where quantification of FES uptake can be difficult because of normal biodistribution, must be evident on the FDG PET scan and CT or MRI. FDG PET imaging will be completed close to the time of the FES PET scan. In select cases where the baseline FDG PET is being repeated at our institution for the purposes of this study it may be completed after the FES PET. The FDG PET will be a clinical 5 axial field-of-view (AFOV) scan from skull base to mid-thigh. Maximum SUV of identified lesions will be calculated and recorded as per standard clinical procedure.

For FDG PET or PET/CT, all patients will fast for at least 4 hours before PET scanning. All patients must have a medical history negative for diabetes and/or a pre-scan serum glucose level less than 200 mg/dL. A dose of 260-370 MBq (7-10 mCi) of FDG will be injected into a peripheral intravenous or central venous catheter. Patients will rest comfortably in a supine position prior to scanning. Imaging will be performed with a commercially made PET/CT scanner operating in the high sensitivity mode. Imaging will consist of a torso survey covering five adjacent 15-cm axial fields of view (FOVs). For the PET/CT device, a low dose CT scan beginning at approximately 60 minutes after FDG injection provides attenuation data for attenuation correction using a non-contrast CT. Emission data will be collected for all five axial FOVs, typically five minutes per FOV.

Emission data will be reconstructed according to standard protocols. Images will be corrected for radioactive decay of the tracer and normalized to the injected dose and body weight, which results in regional standardized uptake values (SUVs): as defined in section 6.10.3.

We emphasize that the baseline FDG PET or PET/CT is part of standard clinical care of metastatic and recurrent breast cancer and will be performed as part of routine clinical care in the vast majority of patients participating in the study. Methodology for FDG PET is included in this section for reference purposes. In some cases, FDG PET or PET/CT will have already been performed at an outside center at the time of enrollment. On rare occasions when patients have been referred to the study with a recent FDG PET scan already completed at an outside site that is unavailable or of insufficient detail for our study, or the patient is unable to obtain a PET scan as part of standard clinical management, we will repeat the FDG PET at the University of Washington as a research scan, however, the imaging procedure will not vary from that of a standard clinical scan.

Patients will undergo a second (and potentially a third in a subset of patients) FDG PET following the same imaging procedure as part of the research study. This second scan will be performed at a time point stipulated by the treatment regimen and may vary from 1 to 12 weeks post therapy start.

6.10.2 [^{18}F]FES-PET Image Assessment

6.10.2.a Summary of Assessment

The FES PET scan will be assessed in multiple ways.

Qualitative FES uptake: all known disease sites positive vs. heterogeneous or absent FES uptake.

Quantitative FES uptake: SUVs in all lesions measured at baseline and repeat scan (and at third scan if indicated)

Quantitative change in FES uptake: percentage and SUV unit change in SUV between baseline and repeat FES PET scan

6.10.2.b Qualitative Visual Assessment

Qualitative (visual) assessment of level of uptake in sites of recurrent breast cancer is performed for all studies. In this analysis, trained observers determine whether FES uptake is present above background at

sites of breast cancer. Active disease sites are identified by the FDG PET scan performed close to the time of FES PET, in conjunction with conventional imaging such as CT, MRI, and bone scan. Active sites of disease are defined as sites with (1) abnormal uptake on FDG PET and (2) either biopsy confirmation or unequivocal correlative findings on at least one other modality. For each site of active disease, two trained observers blinded to the clinical data, but with any available FDG PET and correlative imaging, will determine whether FES above background levels is present or absent. Differences between observers will be resolved by consensus.

6.10.2.c Quantitative Assessment

FES static survey images will undergo region of interest (ROI) analysis as follows: Lesion location will be identified using FES scans along with FDG scans with reference to CT or ultrasound. All patients will have undergone clinically motivated FDG PET scans for staging prior to FES imaging. CT, MRI, ultrasound or bone scan will be available on all patients with suspected metastatic disease. Although FES and FDG imaging will be performed on separate days, studies will be performed with the patient in the same position and images will be co-registered using common anatomic landmarks such as the lung outline, cardiac blood pool, liver contour, and body outline. In this way, regions can be drawn on the FDG images and transferred to the FES images for quantitative analysis which is important in identifying tumor locations, especially in ER-negative tumors which are not visualized on FES imaging. For each disease site, a set of 1.5 cm diameter regions on three adjacent planes with the highest lesion FES uptake will be drawn to determine maximal FES uptake. Up to the 10 largest sites seen on the static torso survey will be quantified. Partial-volume correction will not be used because only sites 1.5 cm or greater diameter will be included in quantitative analysis.

FES uptake will be quantified using the standard uptake value (SUV, defined below). For each site, we will record the average and maximum SUV within the ROI set for each site of disease.

For each subject, we will calculate the average and range of FES uptake values for active disease sites. The FES uptake will be compared with subsequent response to endocrine therapy and TTP.

FDG studies will also be quantified for uptake in a similar fashion. On FDG studies, simple SUVs will be generated using the average tissue activity over the imaging period, which are typically 45 and 75 minutes after injection. We will record the maximum FDG SUV for each site of

disease. Uptake at tumor sites using the standardized uptake value (SUV) is calculated as:

$$SUV = \frac{\overline{C_t}}{ID / wt}$$

where C_t is the average tumor uptake after injection for the period of the static imaging for torso surveys (MBq/mL), ID is the injected dose (GBq) and wt is the patient's weight (kg). Note that either standard or metric units can be used to calculate SUV as long as only one type of unit is used for each calculation.

6.10.3 Biologic Correlates

Optional blood sample may be collected at the time of the FES PET scan procedure for determination of serum hormone levels (estradiol, estrone, follicle-stimulating hormone, sex hormone binding globulin, testosterone, free testosterone).

Patients participating in this study will have undergone clinical biopsy of the primary tumor and/or metastasis for which ER, PgR, and HER2 expression will be determined by IHC. Clinical records of these assays will be reviewed, and the assays themselves may be reviewed as needed.

On patients for whom archival biopsy material can be obtained, additional assays may be performed, and may include repeat and standardized ER, PR, and HER2 assays.

Patients with metastatic or recurrent disease may consent to optional research biopsies of the tumor performed at the baseline, i.e. prior to start of the selected endocrine targeted therapy, and after 1-12 weeks on therapy. Obtained tissues will be analyzed for a baseline expression and post-therapy changes in ER, PgR, HER2, AR, EGFR and VEGF using IHC methods. Some samples will be assayed for intra-tumor steroid hormone levels (estradiol, estrone, testosterone, dihydrotestosterone) using IHC and/or mass spectrometry techniques.

In some patients undergoing biopsy, a whole blood sample will be drawn during the same time point to allow correlation of steroid hormone levels in serum with levels in biopsy tissue.

Assays for ER and PgR will be read using the Allred scoring system, which provides a semi-quantitative and qualitative assessment of tumor expression levels (Harvey, 1999). IHC is the primary assay methodology for HER2. Scoring of HER2 is done by assessing intensity of the membranous stain (0, 1+, 2+, 3+). Scores of 0 and 1+ are considered negative and score of 3+ is considered positive. Score of 2+ is considered indeterminate and will be validated by fluorescence *in situ* hybridization

(FISH) (HER-2 DNA probe kit; Abbott Molecular Inc., Des Plaines, IL)(Yaziji, 2004). Analysis of HER2 will be done using information from the IHC assays and the FISH confirmation when required.

Measurement of these tumor-associated indices may enhance the predictive value of PET FES, provide information to better understand mechanisms of resistance to endocrine therapy, and eventually determine which patients are likely to appreciate clinical benefit as a result of endocrine therapy, even in the absence of an objective response.

6.11 STUDY CALENDAR

Baseline evaluations are to be conducted prior to administration of protocol therapy, typically within 30 days. Baseline FDG PET/CT scan will be obtained near the time of the [^{18}F]FES-PET/CT scan, typically within 14 days. Procedures are outlined below in Table 6.2.

Table 6.2. Study Calendar.

	Pre-Study	Imaging 1	Rx 1	Imaging 2[5]	Rx 2	Imaging 3[6]	Follow up
Informed Consent	X						
Demographics	X						
Pregnancy Test[1]	X						
Vital Signs[2]		X		X		X	
Research lab tests[3]		X		X		X	
ER, PgR, HER2[4]	X						
FDG PET/CT[5,6,7]	X			X		X	
[^{18}F]FES injection and PET[5,7]		X		X		X	
Collect serum hormone levels[8]		X		X		X	
Adverse Event Evaluation		X (0-24hr)		X (0-24hr)		X (0 - 24hr)	
Optional tumor tissue biopsy and blood draw[9]		X		X			

[1] If patient is pre-menopausal, and birth control use is not confirmed
[2] Vital signs include heart rate and blood pressure
[3] Additional blood tests to measure hormone levels (e.g. estradiol, estrone, testosterone, DHT, SHBG) or other hormone related measures might be performed in some patients depending on the treatment regimen.
[4] Pathology results from primary or metastatic biopsy will be reviewed and additional pathology tests may be performed on some patients depending on the treatment regimen.

[5] FES PET/CT and FDG PET/CT will be done at 1-12 weeks after the Imaging 1 time point depending on the treatment regimen.
[6] Repeat FDG PET may be omitted in patients on SERD therapies.
[7] FES PET/CT and FDG PET/CT may be done at 1-12 weeks after the Imaging 2 time point depending on the treatment regimen.
[8] Optional blood sample for estradiol, estrone, follicle-stimulating hormone, sex hormone binding globulin, testosterone and free testosterone serum levels.
[9] In patients consented to optional biopsies, obtain metastatic or recurrent tumor tissue from an FDG and/or FES avid site after completion of PET imaging but prior to start of the endocrine targeted therapy. Repeat the biopsy from the same site after 1-12 weeks on treatment. (Where available and medically appropriate, a clinical or research biopsy that involved sampling of a relevant lesion within 6 months prior to the enrollment may be utilized in place of the baseline optional biopsy.) A blood draw done during the same time point will accompany some biopsies.

6.12 MEASUREMENT OF EFFECT

For the purposes of this study, the outcome measured will be the change in FES and FDG PET measures between the serial sets of scans. Response evaluations will not be evaluated as part of this study, though the information may be recorded if it is collected for treatment studies the patient may be participating. Patients may also be followed for up to 20 years for disease progression and overall survival.

6.12.1 Time to Progression

Time to progression (and progression-free survival) will be measured as the time from the start of endocrine therapy to the time the patient is first recorded as having disease progression. If a patient never progresses while being followed, the patient will be censored at the time he/she terminates follow-up or by date of disease progression or death.

6.13 DATA REPORTING/ REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting are described in Section 6.8.

6.13.1 Data Reporting

The Principal Investigator is responsible for maintaining complete and timely, HIPAA-compliant electronic records of clinical and image datasets.

6.14 STATISTICAL CONSIDERATIONS

6.14.1 Analysis Plan

The primary application will be pilot studies with enrollment of about 20 patients, in which the in vivo effects of endocrine-targeted therapy are measured by serial FES PET. The primary endpoints will be quantitative and qualitative measures of FES uptake, and measures of change in FES uptake (Section 6.10.2). Additionally, patients will be followed for disease progression. Associations among FES and FDG PET measures, progression, and biological correlates (Section 6.10.3) may be examined in descriptive analyses.

An expected primary analysis is to examine whether FES uptake changed on average between serial assessments. This may be assessed by a one-sample test of the percent change in FES SUV. For example, a one-sided test at the 0.05 level of significance could evaluate whether the average

percent change in FES SUV was greater than 20%, considered a biologically significant change. In serial FES SUV measurements of heavily-pretreated breast cancer patients, change in FES SUV was approximately normally distributed with a standard deviation of about 25 percentage points, for both patients treated with blocking therapy (tamoxifen or fulvestrant) or non-blocking therapy (aromatase inhibitor). Using this standard deviation and n=20 to test whether the average change was greater than 20%, the power would be 0.97 if the true change is 40%, and 0.85 if the true change is 35%. To conclude only that the average percentage change was different from zero (using a two-sided test at the 0.05 level of significance), power would be 0.95 to detect a true average change of 20% (and 0.85 if the true standard deviation were 30 percentage points).

Other analyses for pilot studies could examine what proportion of patients experienced a threshold in percentage change, or surpassed a targeted follow-up FES SUV value. These percentages will be reported using a 90% Wilson score binomial confidence interval (Agresti, 1998). Width of these confidence intervals for pilot studies of n=20, n=25, and n=30 are given in Table 6.3 for a range of observed percentages.

Table 6.3: 90% Wilson score binomial confidence intervals (CIs) by percent observed and sample size.

observed percentage (CI) for n=20	observed percentage (CI) for n=25	observed percentage (CI) for n=30
0.1 (0.03, 0.26)	0.12 (0.05, 0.27)	0.1 (0.04, 0.23)
0.2 (0.09, 0.38)	0.20 (0.10, 0.36)	0.2 (0.11, 0.34)
0.3 (0.16, 0.48)	0.32 (0.19, 0.48)	0.3 (0.18, 0.45)
0.4 (0.24, 0.58)	0.40 (0.26, 0.56)	0.4 (0.27, 0.55)
0.5 (0.33, 0.67)	0.48 (0.33, 0.64)	0.5 (0.36, 0.64)

6.14.2 Expected Gender, Ethnic and Racial Composition

Breast cancer is a disease of adults with low frequency of incidence in males. We have not excluded males from participation in our studies and will continue this policy but anticipate that none will enroll due to the rarity of presentation.

Table 6.4. Planned Enrollment.

TARGETED/PLANNED ENROLLMENT: Number of Subjects (*must provide actual numbers. I.e. no range*			
Ethnic Category	**Sex/Gender**		
	Females	Males	Total
Hispanic or Latino	3	0	3
Not Hispanic or Latino	47	0	47
Ethnic Category Total of All Subjects*	50	0	50
Racial Categories			
American Indian/Alaska Native	1	0	1
Asian	8	0	8
Native Hawaiian or Other Pacific Islander	1	0	1
Black or African American	7	0	7
White	33	0	33
Racial Categories: Total of All Subjects *	50	0	50

Males will not be excluded; however, breast cancer is rare in males.

6.14.3 Stratification Factors

Not Applicable

6.14.4 Reporting and Exclusions

6.14.4.a Evaluation of Toxicity: All patients will be evaluable for toxicity for 24 hours after each [^{18}F]FES PET.

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