FRED HUTCHINSON CANCER RESEARCH CENTER
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE

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

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ABBREVIATIONS

FES or F18-FES	[^{18}F]-fluoroestradiol (FES)
FDG or F18-FDG	[^{18}F] -Fluorodeoxyglucose (FDG)
PI	Principal Investigator
PFS	Progression-free survival

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1.0 GENERAL INFORMATION

This is a clinical research protocol and will be conducted under appropriate IRB review. Research procedures will follow all applicable sections of the Code of Federal Regulations, including CFR 21 Part 312.

This clinical study will investigate the utility of [18F]FES in patients with estrogen receptor (ER) positive, primary, recurrent or metastatic, breast carcinoma. [18F]FES PET has been validated as a measure of estrogen receptor expression. This trial is also designed to test the safety of [18F]FES. In addition, this study will obtain data on the value of [18F]FES to predict and evaluate response to endocrine-targeted therapies as a measure of ER expression in this patient population. [18F]-fluoroestradiol (FES) is a sterile injectable radiopharmaceutical designed for imaging estradiol binding to estrogen receptors (ERs) in vivo. FES is currently approved for detection of estrogen-receptor-positive lesions.

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]-16-fluoroestradiol. All patients will undergo a pre- and post-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. 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.

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. 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.

The FES uptake from images will be analyzed qualitatively by visual assessment and quantitatively using a standard uptake value (SUV). 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.

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.

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 [18F]FES imaging will help us design therapeutic trials for better treatment outcomes. We also expect that repeat [18F]FES PET imaging shortly after the start of endocrine-targeted therapy will reflect the pharmacodynamics of endocrine targeted therapy.

1.1 Protocol Title

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

1.2 Sponsor Information

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2.0 INTRODUCTION TO THE PROTOCOL

2.1 Introduction

This clinical study will investigate the utility of [18F]FES in patients with estrogen receptor (ER) positive, primary, recurrent or metastatic, breast carcinoma. [18F]FES PET has been validated as a measure of estrogen receptor expression. This trial is also designed to test the safety of [18F]FES. In addition, this study will obtain data on the value of [18F]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.

2.2 Background

This single-site study will investigate the ability of [18F]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.

Invasive breast carcinoma: Each year, invasive breast cancer will be diagnosed in over 250,000 women and account for over 40,000 deaths in the United States (1). Advances in diagnosis and therapy have resulted in increased survival for early breast cancer (1-3). This increase in breast cancer survival may result in more patients diagnosed with advanced or metastatic breast cancer. Individualized medicine tailoring therapy to patients shows promise to care for this disease (4).

Invasive breast carcinoma and hormone receptors: The incidence of hormone receptor positive disease is approximately 79% (1, 2). Physicians generally choose endocrine-targeted therapies as first-line treatments for patients with ER+ advanced disease due to improved tolerability as well as ease of administration. 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 standard of clinical care (5). Knowledge of hormone receptor

Treatment of breast carcinoma: Treatment selection for breast cancer poses several challenges. Not all patients will respond to first-line endocrine targeted therapy due to intrinsic endocrine-therapy resistance mechanisms as well as tumor heterogeneity. 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. Not all metastatic breast cancer cells will have the same level of estrogen receptor (ER) expression) and ER expression at one site does not guarantee expression at all sites (6). In addition, there are no current methods in standard practice to inform on either of these. This 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 (7). 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.

Recent advances in endocrine therapy: A significant advance in the management of ER+ metastatic breast cancer was the introduction of aromatase inhibitors (AI) (8-10). 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 (8, 10-

12). In addition, Fulvestrant, a selective estrogen receptor down-modulator (SERD, given IM) is active in metastatic disease and may have a particular benefit in de novo disease (13-15). Oral SERDs are in development, with the assistance of FES for pharmacodynamic imaging (16).

The synergistic addition of CDK 4/6 inhibitors and other molecularly targeted agents (mTOR, PARP inhibitors, PI3K inhibitors) has resulted in improved response, duration of response and survival in first and second line settings (17-20). FES measures ER expression, and could measure cross talk (21).

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 (22). Clinical experience suggests a primary role for this strategy in patients who have a significant disease-free interval on a hormonal adjuvant therapy. For post-menopausal women with advanced or metastatic hormone receptor positive (HR+) disease, whose disease is considered treatable but not curable, the initial standard of care treatment is aromatase inhibitors (AIs), with or without CDK (cyclin-dependent kinase) 4/6 inhibition (23). Upon progression, salvage endocrine therapy with molecularly targeted agents, or chemotherapy is indicated (24). Recent Phase III trials combining later-line endocrine therapy with a targeted agent, such as palbociclib, alpelisib or everolimus, have demonstrated considerable improvement in outcome (17, 25, 26) over endocrine therapy alone. Clinical studies have shown promising results when combining endocrine therapy with HDACi, including exemestane with entinostat (27), tamoxifen with vorinostat (28) and a randomized phase III study (E2112) of endocrine therapy plus entinostat/placebo in patients with hormone receptor-positive advanced breast cancer (29). Currently active investigational protocols will determine the optimal management of premenopausal women with hormonal therapy. 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.

2.2.1 Preclinical Data

Nonclinical Pharmacology of FES and Estradiol: The extensive body of literature on the pharmacology and toxicity of fluoroestradiol and of estradiol is summarized below. Estradiol is a naturally occurring substance with biochemical and pharmacologic properties nearly identical to FES. It is important to interpret toxicity data for FES relative to reported toxicity for estradiol in the context of the intended use of FES as a single-dose-administration agent for diagnostic imaging. In this setting, FES reaches physiologic levels (i.e., greater than post-menopausal levels) only on a transient basis. This must be viewed in the context of many years of exposure to physiologic levels of estradiol in most women.

Pharmacokinetics and Metabolism of FES in Animals: The pharmacology of FES has been studied in rats. In rats, FES is rapidly metabolized to more polar species19. By 60 minutes after injection, less than 15% of circulating radioactivity is due to [18F]FES; the remainder is metabolites. Injection of blood from rats obtained 2 hours after injection into different rats showed that the metabolites did not accumulate in ER-rich tissues, such as the uterus, that could be blocked by estradiol19. This suggests that the metabolites are conjugates or other species that do not bind to ER, as compared to unconjugated oxidation products such as the estrone, which would be expected to bind to ER.

Immature Sprague-Dawley female rats received a single injection of [18F]FES and were sacrificed at 30, 60, and 120 min after injection. The results of tissue distribution assays are presented in Table 4.1 as percent injected dose per gram13. [18F]FES was found to have high binding selectivity to target estrogen receptor rich tissues.

Nineteen mature female rats with 7,12-dimethylbenz[a]anthracene (DMBA) induced mammary tumors received a single injection of [18F]FES, and tissue and tumor uptake was measured at 3 hours post injection as % injected dose per gram of tissue and tumor to blood and tumor to non-target tissue ratios. The correlation between [18F]FES and ER content at 3 hours was poor19. However, VanBrocklin et20 al studied 69 female SpragueDawley rats age 22 – 30 days that were injected with 50 µCi of [18F]FES and sacrificed at one-hour post injection to measure alphafetoprotein (AFP) serum concentration and determine the effects of AFP binding on blood activity levels of [18F]FES. A strong positive correlation was seen between serum AFP concentration and [18F]FES blood activity levels, suggesting that the anticipated correlation between uptake of the tracer and ER content may be compromised by the presence of unbound metabolites in the blood and by endogenous molecules that possess high affinity binding sites for phenolic steroids including estrone and estradiol, such as AFP in rats or SHBG in humans20. Time activity curves

for animal models are not directly applicable to human uptake for [18F]FES due to the lack of sex hormone binding globulin (SHBG) in rats.

Toxicology: Multiple studies support the role of estradiol and its catechol estrogen metabolites as weak mutagens and carcinogens in in vivo and in vitro experiments. However, these data, combined with the recent preclinical toxicology and mutagenicity work reported above, also suggest a low frequency of mutagenic events related to estradiol, even with high concentrations and repeated exposures. [18F]FES is therefore extremely unlikely to pose a mutagenic or carcinogenic threat in single-dose exposures such as those required in FES PET. Studies have detected low level alterations to DNA; however, these changes occurred with chronic exposure to estradiol concentrations of 25 μg/ml or greater. This level is 2 – 3 orders of magnitude greater than FES levels encountered in PET, and exposure for PET imaging is transient. The mass of drug used in the FES imaging studies is well below any levels where genotoxicity was observed.

2.2.2 Clinical Data to Date

Imaging of ER with [18F]FES: PET ER imaging using [18F]-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 (30-32). FES-PET evaluates multiple tumor sites simultaneously and, thus, demonstrates the tumors' ability to concentrate estrogen over the entire body. Furthermore FES positron emission tomography (with either PET or PET/CT) measures the heterogeneity of ER expression and function in metastatic disease (33-35). 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 (36). We also showed that the level of FES uptake as an in vivo indicator of ER function predicted response to endocrine therapy with selective ER modulators (SERMs) or aromatase inhibitors (AIs) in first-line therapy or salvage settings (37-40). Typically, a higher tumor SUV was noted in responders compared with non-responders. FES uptake, like IHC, identifies patients with tumors that lack ER expression. This is helpful in distinguishing ER+ tumors that may respond to endocrine therapy from those that do not benefit from such therapy. Serial FES-PET studies show the persistence or loss of ER expression over time in vivo, a potentially useful biomarker to determine when endocrine therapy is likely to be ineffective at controlling tumor growth, and are less invasive, more comprehensive than repeated tissue sampling. In this study we will test FES predictive capability in patients undergoing endocrine therapy of breast cancer, and also to test the impact of endocrine treatments on ER expression and/or ER blockade.

We have examined the role of FES in 47 heavily pretreated patients (39): 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.

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, molecular targets including PI3K and ESR1 mutations. 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.

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

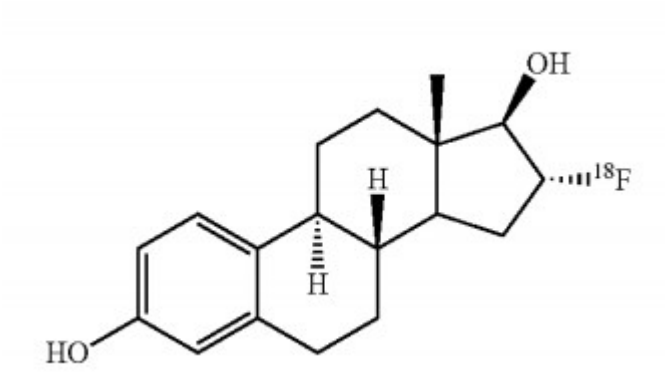
taking TAM showed complete tumor blockade (FES SUV ≤ 1.5 after treatment), compared to 4/11 of FUL patients (p=0.009). Other studies have also shown that FES PET can measure estrogen receptor suppression (16). We concluded that molecular imaging can assess the in vivo pharmacodynamics of targeted agents, and may give insight into the activity of established therapeutic agents.

Phase II trial: Twenty-three patients with ER+/HER2- metastatic breast cancer with prior clinical benefit from endocrine therapy but later progression on aromatase inhibitor (AI) therapy were included in a Phase II trial of vorinostat to restore endocrine sensitivity in ER+/HER2- metastatic breast cancer, with molecular imaging correlates. 18F-FES PET and 18F-Fluorodeoxyglucose (18F-FDG) PET scans were performed at baseline, week 2, and week 8. Simultaneous HDACi and AI dosing in patients with cancers resistant to AI alone was promising with clinical benefit (6+ months without progression) in 4 of 10 evaluable patients. Higher 18F-FES-PET uptake identified patients likely to benefit from combination therapy, but vorinostat did not appear to change ER expression (results submitted for publication).

2.3 Study Agent

The [18F]fluoroestradiol is a sterile, IV injectable solution with a volume of ≤ 20 ml containing 0.15 M phosphate buffered saline: < 15% ethanol (v:v). The injected dose of [18F]FES is generally 6 mCi (185 MBq) with an allowable range of 3 to 6 mCi of [18F]fluoroestradiol. The drug product solution is stored at room temperature in a gray butyl septum sealed, sterile, pyrogen-free glass vial with an expiration time of 8 hours. The mass of injected drug is ≤ 5 µg (≤ 17 nmol) of FES.

Chemically, FES is [18F]16α-fluoro-3,17β-diol-estratriene-1,3,5(10). The molecular weight is 289.37, and the structural formula is:



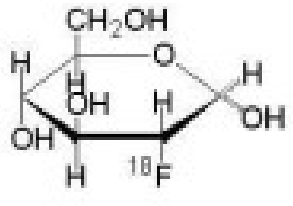
FES is a sterile, clear, colorless solution for intravenous injection, with an osmolarity of 340 mOsm. Its pH ranges between 4.5 to 7.0. The composition of the final product in 40 mL solution is fluoroestradiol no more than 5 µg, fluoroestradiol F 18 148 MBq/mL to 3,700 MBq/mL (4 mCi/mL to 100 mCi/mL), sodium ascorbate 0.44% w/v in sodium chloride 0.9% w/v, and ethanol no more than 3.2% w/v.

2.4 Dose Rationale

The suggested dose is 220 MBq. 222 MBq is the maximum recommended dose in the IB and has been demonstrated to be well-tolerated in terms of toxicity, while providing an accurate image.

2.5 Other Agents

18F-Fluorodeoxyglucose (18F-FDG) Injection is a positron emitting radiopharmaceutical that is used for diagnostic purposes in conjunction with positron emission tomography (PET) imaging. The active ingredient 2-deoxy-2-[18F]fluoro-D-glucose has the molecular formula of C6H11 18FO5 with a molecular weight of 181.26, and has the following chemical structure:



Fludeoxyglucose F 18 Injection is provided as a ready to use sterile, pyrogen free, clear, colorless citrate buffered solution. FDG scans are used as a comparator for this trial. Patients will have a clinical FDG PET scan prior to the start of treatment as part of their standard care. 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).

Risks: Hypersensitivity reactions with pruritus, edema and rash have been reported in the postmarketing setting. FDG will increase the risk of secondary cancer.

2.6 Risks/Benefits

Very few adverse events been reported for diagnostic [18F]FES administration at the strength described for this study in 267 patients studied at the University of Washington. Patients have experienced pain and bruising at the injection site, and one patient reported a temporary slight change in taste. Thus few adverse effects are expected as a result of the administration of [18F]FES, except for discomfort at the injection site. The [18F]FES dose is slightly less than recommended safe oral dose assuming an oral conjugated estrogen dose of 2.5 mg in one day.

The IB also identifies potential risks, which have not been observed in patients receiving FES, but are theoretically possible based on the chemistry and pharmacology of FES: Tissue Injury, infection, extravasation of dose, allergic reaction, nausea, vomiting, anorexia, uterine bleeding, and secondary cancers due to radiation exposure.

FDG PET, blood draws, and biopsies are standard-of-care procedures. FDG PET has additional risks of pruritus, edema, and rash, occurring in less than 1% of patients. The blood draw may briefly cause patients to feel faint, lightheaded, nauseated, mild pain, bleeding, bruising and infection at the site of the needle insertion. Biopsies may cause pain, bleeding at the biopsy site, tenderness, scarring, or infection.

Carcinogenesis has not been observed in animal or clinical trials. Synthetic estrogens such as 2- fluoroestradiol and 4-fluoroestradiol are poor carcinogens in the same animal model systems because the fluorine blocks metabolism when substituted in these positions. [18F]FES is labeled with fluorine at the 16 position of the estradiol. Administration of [18F]FES as described herein, for up to four PET scan procedures, results in intermittent and vastly reduced overall estrogenic exposure compared to regimens known to cause cancer in animals.

FES imaging will not be used to direct therapy. It is not expected that patients on this trial will derive a benefit, this trial is intended only to expand the body of knowledge on FES imaging. The contribution to the body of knowledge for the use of FES is expected to far outweigh the minor risks of FES administration.

3.0 OVERVIEW OF CLINICAL TRIAL

3.1 Study Objectives

3.1.1 Primary Objectives

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

3.1.2 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.

3.2 Study Population

This study focuses on patients with estrogen receptor (ER) positive, primary, recurrent or metastatic, breast carcinoma. Further detail on the subject population can be found in the inclusion and exclusion criteria listed under section 7.

The population for this study will be recruited from patient rosters in other studies that require a FES scan as a research procedure.

3.3 Study Design

This clinical study will investigate the utility of [18F]FES in patients with estrogen receptor (ER) positive, primary, recurrent or metastatic, breast carcinoma. [18F]FES PET has been validated as a measure of estrogen receptor expression. This trial is also designed to test the safety of [18F]FES. In addition, this study will obtain data on the value of [18F]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 [18F]FES imaging will help us design therapeutic trials for better treatment outcomes. We also expect that repeat [18F]FES PET imaging shortly after the start of endocrine-targeted therapy will reflect the pharmacodynamics of endocrine targeted therapy.

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]-16-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 inadequate image quality or 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 indicated 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.

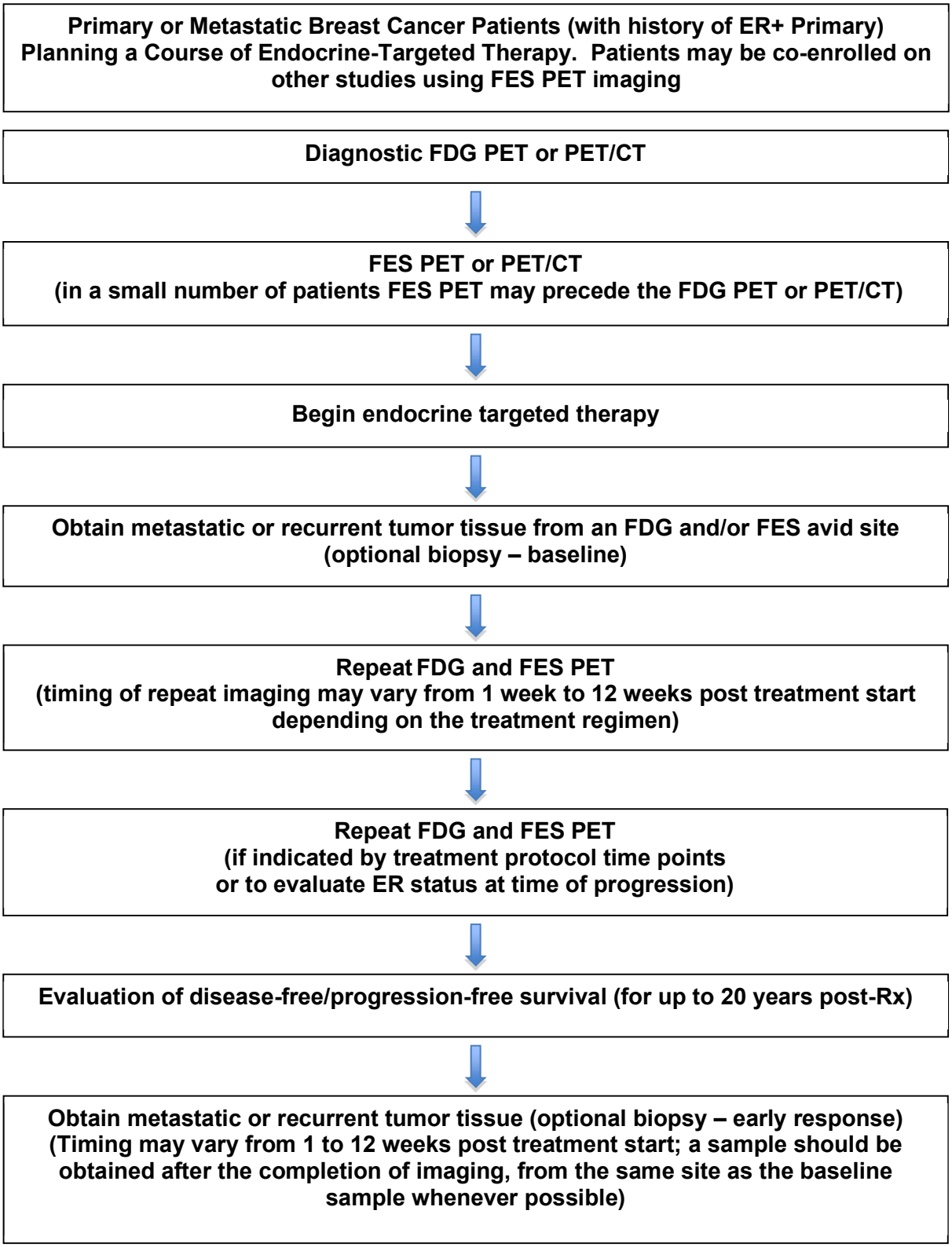
The FES uptake from images will be analyzed qualitatively by visual assessment and quantitatively using a standard uptake value (SUV). Serial measurements of hormone levels in peripheral blood may be used with some treatment regimens to allow determination of efficacy of endocrine therapy. ER, PR and 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 [18F]FES PET will not be used to direct therapy. Treatment regimens are chosen by the referring oncologist prior to the [18F]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 [18F]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.

Below is an outline for this schema:



3.3.1 Primary Endpoint

The primary endpoints will be quantitative and qualitative measures of FES uptake, and measures of change in FES uptake between pre-therapy and post-therapy scans

3.3.2 Secondary Endpoint

Progression-free survival.

3.4 Estimated Accrual

We anticipate that we will enroll up to 20 patients in this pilot study.

4.0 SAFETY CONSIDERATIONS

4.1 Stopping Rules

Participant involvement will be concluded if any AEs possibly related to the IP occur. The principal investigator will exercise their discretion in determining which AEs may be related to the IP. This excludes mild pain, bruising, or controllable bleeding at the injection site, which is a common and expected risk for IV imaging products.

5.0 BRIEF DESCRIPTION OF MANUFACTURING PROCESS FOR F-18 FES

This product is manufactured under IND# 101203. The chemical precursor for [18F]FES is provided in house or by Advanced Biological Compounds 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 assessed by a radiochemist for quality. The quality control tests that must be passed prior to release of the product [18F]FES for injection include the pH, radioactive purity, radiochemical purity, specific activity, sterilizing filter integrity, tests for Kryptofix, acetonitrile, endotoxins and particulates. Acceptance criteria are detailed in the CMC document. The [18F]FES dose is drawn up into a syringe, assayed for mCi at time of injection, labeled and administered to the research subject.

6.0 SUBJECT ELIGIBLITY

6.1 Inclusion Criteria

- Adult, non-pregnant patients with biopsy-proven or clinically obvious primary, recurrent or metastatic breast cancer
- 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.
- At least one site of disease 1.5 cm or greater is needed to meet the spatial resolution limits of PET imaging.
- 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.
- 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.
- Patients must be willing to undergo serial imaging procedures.
- Patients must agree to allow access to clinical records regarding response to treatment and long term follow up.
- Ability to understand and the willingness to sign a written informed consent document.

6.2 Exclusion Criteria

- An inability to lie still for the tests
- Individuals weighing more than 300 lb. (this is the weight limit of the scanner table)
- Pregnant or lactating. Women of childbearing potential with either a positive or no pregnancy test at baseline are excluded.
- 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).
- Use of tamoxifen, faslodex, DES or any other ER blocking agent < 6 weeks or chemotherapy < 3 weeks prior to imaging scan.
- Unwillingness or inability to give informed consent.
- Uncontrolled diabetes mellitus (fasting glucose > 200 mg/dL)
- Adult patients who require monitored anesthesia for PET scanning.

7.0 SUBJECT REGISTRATION

Subjects will be registered by the Fred Hutch/UW Study Coordinator and entered into the Clinical Trials Management System (CTMS). A complete, signed, study consent and HIPAA consent are required for registration.

8.0 TREATMENT PLAN

Treatment will be administered on an outpatient basis. Reported adverse events are described in section 13.0 and potential risks are described in section 2.6. There will be no dose modifications for FES administration. No investigational or commercial agents or therapies will be administered in this protocol with the intent to treat the patient's malignancy.

8.1 Treatment Plan Overview

Figure 6.1 Outline (Schema) of the [18F]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.

Initial visits prior to [18F]FES PET: Patients who are identified as potential candidates for the [18F]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 [18F]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, 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. Patients without a clinical evaluation in the last 30 days are unexpected; in these instances a clinical evaluation will be completed.

Day of [18F]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 [18F]FES for metabolite analysis in selected patients. Heart rate and blood pressure will be measured after the FES injection.

After the [18F]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 [18F]FES PET will be permitted to enter the study, if an additional endocrine targeted treatment is planned.

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.

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.

Optional Biopsy: 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.

Week 1 to 20 years after [18F]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.

A study calendar is included in Appendix A.

8.1 Administration of IP (FES PET)

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 [18F]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 [18F]FES administration. A dose of nominally 220 MBq (6 mCi with a range of 110 to 220 MBq = 3 to 6 mCi) of [18F]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 [18F]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 13.0, Adverse Events).

8.2 Administration of FDG PET

In most cases, a standard clinical diagnostic FDG PET or PET/CT scan will be obtained prior to the [18F]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.

8.3 Concomitant Medication and Supportive Care Guidelines

Because interaction of < 5 µg of [18F]FES with other concomitantly administered drugs through the cytochrome P450 system or other pathways is extremely unlikely, this section is not applicable. The infusion and imaging procedure will be terminated in any patient who exhibits adverse reactions as described in section 4.

8.4 Duration of Therapy

As this is an observational study, the results of [18F]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.

8.5 Duration of Follow-Up

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

8.6 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. FES administration will be stopped if there are any signs of toxcicity.

8.7 End of Treatment (EOT) Visit Schedule and Procedures

EOT visits will not be used on this trial.

9.0 SUBJECT EVALUATION

9.1 On-Study Clinical Evaluations

To establish a baseline, this study plans to pull data from recent clinic visits for patients matching the eligibility criteria, if the visits occurred within the last 30 days. If one is not available, clinical evaluations will take place proceeding both the first and second FES scans (and the third, if applicable). These visits should occur as close to the FES scan as possible but must be within 30 days (+/-) of the FES scan. The following tests and procedures would be completed, although not all tests may be done at each visit;

- Physical Examination

- Vitals
- Labs:
    - Hematology: CBC, differential, platelets
    - Serum Chemistries: including calcium, magnesium, SGOT, SGPT, alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, electrolytes, and glucose

Prior to FES administration, the following are completed:

- A whole blood sample may be taken (10mL) (optional)
    - 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).
- Heart rate and blood pressure

Following FES Administration, the following are completed:

- For patients able to have a second venous access, venous blood samples may be collected at 5, 20, and 60 minutes after collection.
- Heart rate and blood pressure

10.0 TOXICITY MONITORING

Both acute and chronic toxicities are recorded and reported to the Sponsor, or its designee, and will be reviewed by the independent DMC at scheduled meetings. Monitoring for acute toxicity takes place during and immediately following IP administration for a period of 2 hours at the study site. Subjects are observed for the development of an immediate localized allergic reaction or anaphylactic reaction during this time. Chronic toxicity is evaluated describe here. Describe acceptable or unacceptable toxicity treatments here.

11.0 SUBJECT DISCONTINUATION OF ACTIVE TREATMENT

Subjects may be removed from this study at any time at their discretion. Subjects may also be removed from this protocol if they develop any untoward side effects from the imaging product.

If a subject withdraws consent to participate in the study or aspects of the study, attempts should be made to obtain permission to record survival data up to the protocol-described end of the subject follow-up period. Survival data are important to the integrity of the final study analysis. Documentation in the medical record should state that the subject is withdrawing from the study and what, if any, selected data the subject will permit the investigator to obtain.

An explanation for discontinuing treatment is recorded for each subject discontinuing treatment in the study source. The Sponsor must be notified immediately if a subject discontinues treatment. All subjects, irrespective of treatment status, will continue to be followed for survival. Treatment in this study must be discontinued for any of the following reasons:

- if the Sponsor decides to stop the study;
- at Investigator's discretion;
- at the subject's request;
- if the subject enrolls in a trial of another investigational agent;
- Any adverse event possibly related to the IP, excluding mild pain, bruising, or controllable bleeding at the injection site
- pregnancy

12.0 CONCOMITANT MEDICATIONS

Because interaction of < 5 µg of [18F]FES with other concomitantly administered drugs through the cytochrome P450 system or other pathways is extremely unlikely, this section is not applicable.

The infusion and imaging procedure will be terminated in any patient who exhibits adverse reactions as described in section 4.

13.0 ADVERSE EVENTS

13.1 Adverse Event

According to ICH guidelines (Federal Register. 1997; 62(90):25691-25709) and 21 CFR 312.32, IND Safety Reports, and ICH E2A, Definitions and Standards for Expedited Reporting, an adverse event is defined as follows:

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

Abnormal laboratory values for laboratory parameters specified in the study should not be recorded as an adverse event unless an intervention is required (repeat testing to confirm the abnormality is not considered intervention), the laboratory abnormality results in a serious adverse event or the adverse event results in study termination or interruption/discontinuation of study treatment.

Medical conditions present at screening (i.e., before the study treatment is administered) are not adverse events and should not be recorded on adverse event pages of the CRFs. These medical conditions should be adequately documented on the subject chart. However, medical conditions present at baseline that worsen in intensity or frequency during the treatment or post-treatment periods should be reported and recorded as adverse events.

13.2 Serious Adverse Event

An adverse event should be classified as an SAE if it meets one of the following criteria:

Table 13.1: SAE Criteria

Fatal	Adverse event results in death.
Life threatening:	The adverse events placed the subject at immediate risk of death. This classification did not apply to an adverse event that hypothetically might cause death if it were more severe.
Hospitalization:	It required or prolonged inpatient hospitalization. Hospitalizations for elective medical or surgical procedures or treatments planned before enrollment in the treatment plan or routine check-ups are not SAEs by this criterion. Admission to a palliative unit or hospice care facility is not considered to be a hospitalization.
Disabling/incapacitating	Resulted in a substantial and permanent disruption of the subject's ability to carry out normal life functions.
Congenital anomaly or birth defect:	An adverse outcome in a child or fetus of a subject exposed to the molecule or treatment plan regimen before conception or during pregnancy.
Medically significant:	The adverse event did not meet any of the above criteria, but could have jeopardized the subject and might have required medical or surgical intervention to prevent one of the outcomes listed above.

13.3 Unexpected Adverse Event

An unexpected adverse event is defined as an event that has a nature or severity, or frequency that is not consistent with the applicable investigator brochure, or the prior medical condition of the subject or other treatment given to the subject. "Unexpected," as used in this definition, refers to an adverse drug experience that has not been previously observed and reported in preclinical or clinical studies rather than an experience that has not been anticipated based on the pharmacological properties of the study drug.

13.4 Monitoring and Recording Adverse Events

All AEs will be assessed by the investigator or qualified designee and recorded in the source documentation. The investigator should attempt to establish a diagnosis of the event on the basis of signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the adverse event and/or serious adverse event and not described as the individual signs or symptoms. The following information should be recorded:

- Description of the adverse event using concise medical terminology
- Description as to whether or not the adverse event is serious, noting all criteria that apply
- The start date (date of adverse event onset)
- The stop date (date of adverse event resolution)
- The severity (grade) of the adverse event
- A description of the potential relatedness of the adverse event to study drug, a study procedure, or other causality
- The action taken due to the adverse event
- The outcome of the adverse event

Because adverse events are not expected for this IP, and have not been seen in our experience with FES, patients with AEs possibly related to the IP will be discontinued. The principal investigator will exercise their discretion in determining the attribution of AEs.

Patients who discontinue the study will be asked if follow-up via telephone is acceptable. If they agree to receive communication from us, we will follow-up with them 30 days following administration of the IP. If any unexpected risks are discovered which may affect patients more than 30 days following administration, all previous participants will be informed.

13.5 Grading Adverse Event Severity

All AEs will be graded in severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. If a CTCAE criterion does not exist, the investigator should use the grade or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the adverse event.

13.6 Attribution of an Adverse Event

Association or relatedness to the study agent will be assessed by the investigator as follows:

- Definite: The event follows a reasonable temporal sequence from exposure to the investigational agent, has been previously described in association with the investigational agent, and cannot reasonably be attributed to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications; AND the event disappears or improves with withdrawal of the investigational agent and/or re-appears on re-exposure (e.g., in the event of an infusion reaction).
- Probable: The event follows a reasonable temporal sequence from exposure to the investigational agent and has been previously been described in association with the investigational agent OR cannot reasonably be attributed to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Possible: The event follows a reasonable temporal sequence from exposure to the investigational agent, but could be attributable to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Unlikely: Toxicity is doubtfully related to the investigational agent(s). The event may be attributable to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Unrelated: The event is clearly related to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.

For general AE assessment, an AE is considered related if it is assessed as definitely, probably, or possibly related; unrelated if it is assessed as unlikely related or unrelated.

13.7 Adverse Event Recording Period

AEs will be monitored and recorded in study-specific case report forms (CRFs) from the time of first exposure to an investigational product in this study. Patients will be contacted 24 hours following IP administration to evaluate for signs of AEs. AEs with an onset date prior to the first exposure to an investigational product will not be recorded, except in the case of clinically significant worsening of the AE during the specified AE monitoring time frame.

13.8 Adverse Event Reporting Requirements

Expedited AE reporting for this study will be completed with the FDA and as required by FDA MedWatch, and the IND sponsor. These requirements are briefly outlined in the table below.

All life-threatening adverse reactions reports are submitted to the sponsor, and to all participating investigators. The sponsor will report all attributable, unexpected life-threatening or otherwise serious AEs to the FDA. A copy of the report is kept on file. If a new risk is identified to be attributable to FES, it will also be reported to the IRB as a protocol and consent change within 10 days.

Table 13.2 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	**Sponsor report to FDA ASAP and within 7 days of discovery of event**	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

14.0 CRITERIA FOR ENDPOINT EVALUATIONS

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.

The FES PET scan will be assessed with:

- 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

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.

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 Ct 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.

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) and liquid biopsy.

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 protein and genomic expression including ER, PgR, and HER2, using IHC methods and PI3K and ESR1 using Oncoplex. 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.

Time to progression (Progression-free survival): 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.

15.0 DATA AND SAFETY MONITORING PLAN

Institutional support of trial monitoring will be in accordance with the FHCRC/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, FHCRC Clinical Research Support (CRS) coordinates data and compliance monitoring conducted by consultants, contract research organizations, or FHCRC employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), FHCRC Scientific Review Committee (SRC) and the FHCRC/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating subjects. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines.

16.0 DATA MANAGEMENT/CONFIDENTIALITY

Source data will include all signs collected during this research. This includes:

- All imaging data, including scan images
- Vitals and physical exams collected at clinic visits
- Labs:
    - Hematology: CBC, differential, platelets
    - Serum Chemistries: including calcium, magnesium, SGOT, SGPT, alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, electrolytes, and glucose

The imaging will be stored on an encrypted hard drive and treated as source. Only the appropriately delegated staff will be given the password to access this data.

The investigator will ensure that data collected conform to all established guidelines. Each subject is assigned a unique subject number to protect subject confidentiality. Subjects will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents.

17.0 STATISTICAL CONSIDERATIONS

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 between pre-therapy and post-therapy scans (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. 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 17.1: 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)

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.

Ethnic Category	**Sex/Gender**		
	Females	Males	Total

Hispanic or Latino	3	0	3
Not Hispanic or Latino	17	0	17
Ethnic Category Total of All Subjects	20	0	20
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	3	0	3
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	1	0	1
White	16	0	16
Racial Categories: Total of All Subjects	20	0	20

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

Patients will be recruited from participants in other studies requiring [^{18}F]FES PET. Over the course of this study, more studies will be added requiring [^{18}F]FES PET, possibly increasing the enrollment target. The IRB will be made aware of changes to enrollment targets.

18.0 INVESTIGATOR OBLIGATIONS

The PI is responsible for the conduct of the clinical trial at the site and is responsible for personally overseeing the treatment of all study subjects. The PI must assure that all study site personnel, including sub-Investigators and other study staff members, adhere to the study protocol and to all applicable regulations and guidelines regarding clinical trials both during and after study completion.

All subjects are informed of the nature of the program, its possible hazards, and their right to withdraw at any time, and each subject signs a form indicating their consent to participate prior to receiving any study-related procedures (see Appendices XXX).

19.0 ADMINISTRATIVE AND REGULATORY CONSIDERATIONS

19.1 Pre-Study Documentation

The following documentation required by the FDA must be received by the Sponsor, or its designee, prior to initiation of the trial: FDA Form 1572; curricula vitae of the PI and all Sub-Investigators; signed Protocol Agreement; copy of the correspondence from the IRB indicating approval of the protocol and Informed Consent Forms, signed by the IRB chairperson or designee; an IRB membership list containing the names and occupations of the IRB members; copy of the Informed Consent Forms that were reviewed and approved by the IRB.

19.2 Study Site Training

Before initiation of the study, the Sponsor, or its designated representatives will review and discuss the following items with the Investigator and clinic staff: the protocol, study procedures, record keeping and administrative requirements, drug accountability, AE reporting, Good Clinical Practice guidelines, CRF/eCRF completion guidelines, monitoring requirements, and the ability of the site to satisfactorily complete the protocol. Additional documents with instructions for study compliance and CRF/eCRF completion will be provided.

19.3 Documentation

The documentation of clinical data must be stored by the Sponsor according to legal requirements. The PI and study staff has responsibility for maintaining a comprehensive and centralized filing system containing all study-related documentation. These files must be suitable for inspection by the Sponsor, the FDA, and/or other applicable regulatory agencies/competent authorities at any time, and should consist of the following elements: subject files (complete medical records, laboratory data, supporting source documentation, and the Informed Consent); study files (the protocol with all amendments, copies of all pre-study documentation, and all correspondence between the Competent Authorities, IRB, site, and Sponsor); and drug accountability files, containing a complete account of the receipt and disposition of the study drug.

19.4 Access to Source Data

The PI will permit the Sponsor's representatives to monitor the study as frequently as the Sponsor deems necessary to determine that protocol adherence and data recording are satisfactory. The CRF/eCRF and related source documents will be reviewed in detail by the Sponsor's representative at each site visit. Only original source documents are acceptable for review. This review includes inspection of data acquired as a requirement for participation in this study and other medical records as required to confirm information contained in the CRF/eCRF, such as past history, secondary diagnoses, and concomitant medications. Other study records, such as correspondence with the Sponsor and the Competent Authorities, and IRB and screening and drug accountability logs will also be inspected. All source data and study records must also be available for inspection by representatives of the FDA or other regulatory agencies.

19.5 Data Collection

Describe how data will be collected.

Electronic case report forms must be completed and submitted for each subject enrolled in the study. Any changes or corrections made to the CRF/eCRF must be subsequently reviewed and signed by the PI. All data fields in the CRF/eCRF must be completed to avoid queries.

19.6 Protocol Interpretation and Compliance

The procedures defined in the protocol are carefully reviewed by the PI and his/her staff prior to the time of study initiation to ensure accurate representation and implementation. Protocol amendments, if any, are reviewed and implemented promptly following IRB and relevant Competent Authorities approval. The Sponsor is responsible for submitting protocol amendments to the FDA as described in 21 CFR § 312.30 (Protocol Amendments) and other regulatory agencies according to national, state or local requirements. The Sponsor, or its designee, is always available to answer protocol- or subject-related questions.

19.7 Study Monitoring and Data Collection

A representative from the Sponsor will visit the study center periodically to monitor adherence to the protocol, applicable FDA regulations and/or other regulatory agencies national, state or local requirements, and the maintenance of adequate and accurate clinical records. Electronic case report forms are reviewed to ensure that key safety and efficacy data are collected and recorded as specified by the protocol. The Sponsor or its designee is permitted to access subject medical records, laboratory data and other source documentation as needed to appropriately monitor the trial.

19.8 Disclosure of Data/Publication

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties other than those noted below is prohibited. Such medical information may be given to the subject's personal physician or to other appropriate medical personnel responsible for the subject's welfare. Data generated as a result of this study are to be available for inspection on request by the FDA or other regulatory agencies, the Sponsor or its designee and by the IRB.

It is anticipated that the final results of this study will be submitted to a peer-reviewed scientific journal. Authorship on such a paper will be acknowledged with customary scientific practice. As such, without the expressed permission of the Sponsor, only clinical Study data relating the Study as a whole will be published. If permission is granted by Sponsor for publication of ancillary data from individual sites, prior to submission for publication of any manuscript or presentation of any poster, presentation, abstract or other written or oral material that describes the results of Study, Institution and/or PI shall provide Sponsor at least 60 days (or as otherwise specified in the sites executed Clinical Trial Agreement) to review any such materials. Such materials shall not divulge any of Sponsor's Confidential Information, and Institution and/or PI shall promptly remove any Confidential Information as requested by Sponsor. If requested by Sponsor, the PI and Institution shall delay the submission of any publication or presentation up to 60 days from the date of Sponsor's request for such a delay. In addition, Sponsor has the right to require that any publication or presentation concerning the Study will acknowledge Sponsor's support.

19.9 Ethical Considerations

The Investigator agrees to conduct this study in accordance with applicable United States FDA clinical trial regulations and guidelines, applicable United States FDA clinical trial regulations and guidelines, the ICH (E6) GCP guidelines, n, the IRB and local legal requirements and with the Declaration of Helsinki (1989). The Investigator will conduct all aspects of this study in accordance with all national, state, and local laws of the applicable regulatory agencies.

19.10 Informed Consent

The PI assumes the responsibility of obtaining written Informed Consent for each subject before any study-specific procedures are performed.

Subjects meeting the criteria set forth in the protocol will be offered the opportunity to participate in the study. To avoid introduction of bias, the Investigator must exercise no selectivity with regard to offering eligible subjects the opportunity to participate in the study. Subjects or parents/legal guardians of all candidate subjects will receive a comprehensive explanation of the proposed treatment, including the nature of the therapy, alternative therapies available, any known previously experienced adverse reactions, the investigational status of the study drug, and other factors that are part of obtaining a proper Informed Consent. Subjects will be given the opportunity to ask questions concerning the study, and adequate time to consider their decision to or not to participate.

Informed Consent will be documented by the use of a written Consent Form that includes all the elements required by FDA regulations and ICH guidelines. The Sponsor or designee will review the informed consent prior to submission to the IRB. The form is to be signed and dated by the subject and by the person who administers the consent process. A copy of the signed form will be given to the person who signed it, the original signed Consent Form will be filed with the subject's medical records, and copy maintained with the subject's study records. The date and time of time of the Informed Consent must be recorded in the source documents.

If an amendment to the protocol changes the subject participation schedule in scope or activity, or increases the potential risk to the subject, the Informed Consent Form must be amended. Any amended Informed Consent must be reviewed by the Sponsor or designee and approved by the IRB prior to use. The revised Informed Consent Form must be used to obtain re-consent from any subjects currently enrolled in the study if the subject is affected by the amendment, and must be used to document consent from any new subjects enrolled after the approval date of the amendment.

19.11 Institutional Review Board/Ethics Committee

The PI will assure that an appropriately constituted IRB that complies with the requirements of 21 CFR Section 56 or written assurance of compliance with ICH (E6) guidelines will be responsible for the initial and continuing review and approval of the clinical study. Before initiation of the study, the PI or designee will forward copies of the protocol and Consent Form to be used for the study to the IRB for its review and approval. A photocopy of the IRB notification of approval must be forwarded to the Sponsor or its designee before any investigational supplies will be shipped to the PI.

The PI or designee will also assure that all changes in the research activity and all unanticipated problems involving risks to human subjects or others will be reported promptly to the IRB, and that no changes will be made to the protocol without prior Sponsor and IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

Copies of all study-related correspondence between the Investigator and the IRB must be provided to the Sponsor, or its designee, by the Investigator. The PI or designee must promptly notify the IRB of any SAE occurring at the site and of any safety reports (e.g., IND Safety Reports) received from the Sponsor, or its designee, and must copy the Sponsor, or its designee on that correspondence.

The Investigator or designee will be responsible for submitting periodic progress reports to the IRB at intervals appropriate to the degree of subject risk involved in the study, but not less than once per year and at the completion or termination of the study.

19.12 Subject Privacy

The Sponsor and the Investigator affirm and uphold the principle of the subject's right to privacy. The Sponsor, it's designates and the Investigator shall comply with applicable national and local privacy laws.

To verify compliance with this protocol, the Sponsor, or its designee, will require that the Investigator permit the Sponsor, or its designee's monitor to review the subject's original medical records. Should access to such medical records require a waiver or authorization separate from the statement of Informed Consent, the Investigator will obtain such permission in writing from the subject before the subject is entered into the study.

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21.0 Appendix

Appendix A: 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:

	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.