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Title:

[^{18}F]Fluoroestradiol-PET/CT Imaging of Invasive Lobular Carcinoma

Matthew F. Covington, MD Principal Investigator

Imaging radiopharmaceuticals to be used:

[^{18}F]Fluoroestradiol ([^{18}F]FES)
IND # 151981

2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG)
ANDA # 204498

University of Utah IRB # 128055

PROTOCOL SIGNATURE

I confirm that I have read this protocol, and I will conduct the study as outlined herein and according to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable ICH guidelines for good clinical practice, and the applicable laws and regulations of the federal government. I will promptly submit the protocol to the IRB for review and approval. Once the protocol has been approved by the IRB, I understand that any modifications made during the course of the study must first be approved by the IRB prior to implementation except when such notification is made to remove an immediate hazard to the subject.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study treatment, the conduct of the study, and the obligations of confidentiality.

Note: This document is signed electronically through submission and approval by the Principal Investigator in the University of Utah IRB Electronic Research Integrity and Compliance Administration (ERICA) system.

1. OBJECTIVES

1.1. Primary Objective for Pilot Phase (Completed as of March 2022 Amendment)

To assess the positive detection rate of invasive lobular carcinoma (ILC) on 18F]Fluoroestradiol-PET/CT (FES-PET/CT).

Primary endpoint:

1) FES-PET/CT will detect at least 80% of histologically proven primary ILC estrogen receptor positive (ER+) tumors. Reference standard is histopathology from biopsy of a primary breast or metastatic lesion demonstrating ILC. The null hypothesis is that 60% of cases will demonstrate positive uptake on FES, equivalent to rates from pooled data for FDG-PET/CT [1-3]. A higher proportion of tumors are expected to demonstrate positive uptake by FES-PET/CT.

1.2. Secondary Objective for Pilot Phase (Completed as of March 2022 Amendment)

To assess FES-PET/CT concordance with ER status from biopsy and presence of inter-tumoral ER heterogeneity.

Secondary endpoints:

1. Rate of estrogen receptor positive (ER+) ILC that does not demonstrate positive FES uptake, defined as focal uptake above background with SUV max of 1.5 or greater.
2. Rate of estrogen receptor negative (ER-) ILC that does demonstrate positive FES uptake, defined as focal uptake above background with SUVmax of 1.5 or greater.
3. Rate of same-patient (inter-tumoral) heterogeneous FES uptake defined as presence of FES uptake with SUVmax of 1.5 or greater in some but not all biopsy proven or suspected metastatic lesions.

To assess for any differences between FDG- and FES-PET/CT uptake.

Secondary endpoints:

1. For cases with both FDG- and FES-PET/CT imaging, evaluate the rate of discordant uptake (FES positive/FDG negative or FES negative/FDG positive). Discordant uptake will be evaluated for biopsy proven primary, any proven or suspected local nodal (axillary, intramammary, internal mammary, supraclavicular) and any proven or suspected distant metastatic lesions. Note that completion of an FDG-PET/CT study is not obligatory for study enrollment, but an optional FDG-PET/CT will be paid for by the study if the patient elects to complete this study, and if not already obtained for clinical evaluation.
2. For cases with both FDG- and FES-PET/CT imaging, evaluate the correlation of lesion uptake between FES and FDG.

1.3. Primary Objective for Expansion Phase (Added as of March 2022 Amendment)

To assess **change in staging** of patients with newly diagnosed ILC (primary study arm, n=40) **based on FES-PET/CT results** per the American Joint Committee on Cancer (AJCC) TNM staging system.
Primary endpoint:

- Overall percentage of change of stage following research FES-PET/CT imaging compared to stage based on standard of care imaging.

1.4. Secondary Objectives for Expansion Phase (Added as of March 2022 Amendment)

To assess whether **quantity of methylated ctDNA** at baseline (primary study arm, n=40) **predicts patient stage at presentation** per the American Joint Committee on Cancer (AJCC) TNM system.
Secondary endpoint:

- MethylPatch PCR ctDNA quantity at baseline as predictor of patient stage at baseline

To assess whether **quantity of methylated ctDNA** at baseline (primary study arm, n=40) **predicts survival at each follow-up interval** starting at 6 months (follow-up performed at 6, 12, 18, 24, 36, 48 and 60 months).
Secondary endpoint:

- Correlation between quantity of methylated ctDNA with survival based on survival analysis at each follow-up period.

To assess whether **heterogeneous FES-PET/CT uptake** at baseline (yes/no) (primary study arm, n=40), defined as abnormal FES uptake in some but not all proven or suspected ILC lesions, **predicts survival at each follow-up interval** starting at 6 months (follow-up performed at 6, 12, 18, 24, 36, 48 and 60 months).
Secondary endpoint:

- Presence of heterogeneous FES-PET/CT uptake at baseline (binary variable/yes/no) as a predictor for survival as assessed by a survival analysis on follow-up assessments.

1.5. Exploratory Objectives for Expansion Phase (Added as of March 2022 Amendment)

Evaluate **rate of complete ER blockade** (yes/no) **on optional post-therapy FES-PET/CT** study (Primary arm, n=10 patients) with ER+ ILC completing an optional FES-PET/CT study 2-4 weeks after therapy onset.
Secondary endpoint:

- Rate of persistent abnormal FES uptake at sites of known or suspected ILC (yes/no).

Evaluate **rate of persistent FES uptake** (yes/no) **on optional FES-PET/CT** (Exploratory Arm 1, n=10) for individuals with known metastatic initially ER positive ILC currently on hormonal therapy.
Exploratory endpoint:

- Rate of persistent FES uptake (yes/no) while on therapy

Assess the **negative predictive value of FES-PET/CT** imaging (Exploratory Arm 2, n=5) for individuals with estrogen receptor (ER) negative ILC as determined by immunohistochemistry from core needle biopsy.
Exploratory endpoint:

- Negative predictive value for negative (no abnormal uptake) FES-PET/CT imaging

Assess whether **heterogeneous FES-PET/CT uptake at baseline** (yes/no) for participants in Primary and Exploratory Arm 2, defined as abnormal FES uptake in some but not all proven or suspected ILC lesions, **predicts development of recurrent disease (yes/no) for patients with no distant metastatic disease at baseline,** during follow-up starting at 6 months (follow-up performed at 6, 12, 18, 24, 36, 48 and 60 months).
Exploratory endpoint:

- Presence of heterogeneous FES-PET/CT uptake at baseline (binary variable/yes/no) and development of recurrent disease at follow-up (yes/no).

To assess whether **heterogeneous FES-PET/CT uptake at baseline** (yes/no) for participants in all study arms, defined as abnormal FES uptake in some but not all proven or suspected ILC lesions, **predicts development of new metastatic disease during follow-up** (yes/no) for patients who have metastatic disease at baseline, during follow-up starting at 6 months (follow-up performed at 6, 12, 18, 24, 36, 48 and 60 months).
Exploratory endpoint:

- Presence of heterogeneous FES-PET/CT uptake at baseline (binary variable/yes/no) and development of recurrent disease at follow-up (yes/no).

Assess whether **quantity of methylated ctDNA at baseline** for participants in Primary Arm and Exploratory Arm 2 **predicts development of recurrent disease (yes/no) for patients with no distant metastatic disease at baseline,** during follow-up starting at 6 months (follow-up performed at 6, 12, 18, 24, 36, 48 and 60 months).
Exploratory endpoint:

- Quantity of methylated ctDNA at baseline as predictor of development of recurrent disease at follow-up (yes/no).

To assess whether **quantity of methylated ctDNA at baseline** for participants in all study arms **predicts development of new metastatic disease during follow-up** (yes/no) for patients who have metastatic disease at baseline, during follow-up starting at 6 months (follow-up performed at 6, 12, 18, 24, 36, 48 and 60 months).
Exploratory endpoint:
Quantity of methylated ctDNA at baseline at baseline as predictor of development of new metastatic disease at follow-up (yes/no).

2. BACKGROUND

2.1. Invasive Lobular Carcinoma

ILC is a distinct molecular and pathologic entity from invasive ductal carcinoma and is the second most common type of breast cancer next to invasive ductal carcinoma, comprising up to 15% of invasive breast cancer cases in the United States [1, 2, 4-6].

2.2. Imaging of Invasive Lobular Cancer

According to the National Comprehensive Cancer Network (NCCN) 2018 guidelines FDG-PET/CT may be performed as an alternative to a contrast-enhanced CT of the chest, abdomen and pelvis and Tc-99m MDP bone scan for evaluation of distant metastatic disease in newly diagnosed stage III breast cancer patients [7]. FDG-PET/CT is usually not obtained for stage I or stage II breast cancer patients as change in patient management is rare [7]. Prior studies have demonstrated FDG-PET/CT can identify sites of unsuspected metastatic disease in newly diagnosed breast cancer patients thereby altering treatment decisions given that palliative management is typical for stage IV disease, whereas neoadjuvant therapy followed by surgery and postoperative radiation may be considered for stage II and operable stage III disease [1, 7-11]. These guidelines consider invasive breast cancer as a single entity and do not consider whether tailoring imaging techniques for subtypes of breast cancer may be beneficial. However, prior research suggests that FDG-PET/CT may be more appropriate as an alternative to CT and bone scan for patients with invasive ductal carcinoma (IDC) rather than invasive lobular carcinoma (ILC) as FDG demonstrates comparatively reduced sensitivity for ILC metastases [1, 2, 6, 12, 13]. Compared to IDC, ILC is more often occult on mammography, ultrasound, and FDG-PET/CT [1-4, 6]; which is of importance for clinical management as ILC is more often multifocal and bilateral compared to IDC

[14]. Clinical breast examination also has lower sensitivity for detection of ILC compared to IDC, even for large tumors, as ILC may be indistinguishable from normal breast tissue on palpation [5, 15].

A prior study evaluating systemic staging of newly diagnosed patients with stage I-III invasive breast cancer found that FDG-PET/CT is 1.98 times less likely to reveal unsuspected distant metastatic disease for women with ILC compared to IDC [1]. In this study, all IDC metastases demonstrated FDG avidity whereas 25% of ILC metastases (3 of 12) were not FDG avid [1]. Detection of local axillary metastatic disease on FDG-PET/CT was also lower for ILC (0 of 146 patients) compared to IDC (7 of 89 patients)[1] despite data from the Surveillance, Epidemiology and End Results (SEER) database demonstrating similar rates for lymph node metastases between IDC and ILC [14]. Another study evaluating FDG-PET/CT for the diagnosis of primary breast cancer found that the false negative rate for detection of ILC by FDG was 65% (15 of 23 cases) compared to 23% for IDC (23 of 97 cases) when matching for tumors of the same size [3]. A final study reported a false negative rate of FDG for ILC detection of 13% (2 of 15 patients) [2]. Mechanistically, ILC may not take up FDG as avidly as IDC due to lower tumor microvascularity, cellular density, proliferation rate, and number of GLUT transporters [2, 6, 12, 16-20]. ILC osseous metastatic disease is also more frequently occult on FDG-PET/CT compared to IDC [3, 12] as ILC osseous metastases are more frequently sclerotic, whereas FDG-PET/CT is more sensitive for lytic osseous metastases. Sclerotic ILC osseous metastases also may be indistinguishable from benign bone islands on CT at initial staging, thereby necessitating biopsy or imaging follow-up for confirmation of osseous metastatic disease [3, 12]. Improved imaging strategies for primary and metastatic ILC are therefore warranted.

Multiple studies have proven the efficacy of FES-PET/CT for imaging evaluation of ER+ invasive breast malignancy (evaluating both IDC and ILC together, with the large majority of cases comprising IDC) but, to our knowledge, no prior study has focused FES-PET/CT evaluation only to cases of ILC, nor have prior studies compared FES-PET/CT directly with FDG-PET/CT for evaluation of newly diagnosed ILC. Given that all prior studies on FES-PET/CT have grouped a small number of ILC cases with a larger number of IDC cases, the imaging performance of FES-PET/CT specifically for ILC is unknown. ILC demonstrates higher rates of ER positivity than IDC with prior studies showing greater than 90% positivity for cases of ILC [1, 6]. Data from the SEER database also shows ILC demonstrates higher overall expression of ER than IDC (ILC 95% positive for ER, n=17,503 vs IDC 74% positive for ER, n=172,379) [14]. FES-PET/CT may therefore be suitable for imaging evaluation of a high proportion of patients with ILC.

2.3. Overview of the PET Radiopharmaceutical [^{18}F]Fluoroestradiol

FES is an emerging PET radiopharmaceutical that utilizes binding of radioactive-labeled estradiol to estrogen receptors, thereby allowing direct imaging of estrogen receptor uptake within breast malignancy [21-47]. Prior studies have shown that FES binding to the ER is essentially equivalent to that of endogenous estradiol and FES uptake on PET

imaging has been shown to correlate with ER expression on in vitro assays such as radioligand binding or immunohistochemistry [43, 46].

According to the Cancer Imaging Program of the National Cancer Institute (CEP-NCI) [^{18}F]Fluoroestradiol Investigator Drug Brochure Version 5 (2017), over 1450 patients have received FES, either under the Radioactive Drug Research Committee program or under an Investigational New Drug (IND) application. FES-PET/CT is increasing our understanding of the role of ER expression in invasive breast malignancies, both for diagnosis and staging of breast malignancy, in addition to informing the use of hormonal therapy versus chemotherapy or other therapies for patients with known breast cancer. In May 2020, FDA approved the use of FES manufactured by Zionexa for detection of estrogen receptor (ER)-positive lesions as an adjunct to biopsy in patients with recurrent or metastatic breast cancer.

As a non-invasive agent, FES-PET/CT allows imaging of the entire body for abnormal estrogen receptor density without requiring a biopsy, thereby facilitating the in vivo assessment of estrogen receptors. FES-PET/CT allows imaging of the entire volume of receptor status within every tumor, thereby avoiding potential sampling error from tissue sampling of only a portion of a tumor and providing a potential route to evaluate for estrogen receptor heterogeneity within a tumor or between sites of primary and metastatic malignancy. Additionally, by providing an imaging biomarker for assessment of estradiol binding to the estrogen receptor, FES-PET/CT assesses the biological activity of the estrogen receptor, which is not possible by immunohistochemistry.

Although beyond the scope of the current study, FES-PET/CT may guide therapeutic decision making by documenting the presence of estrogen receptor heterogeneity in primary and metastatic invasive breast cancer lesions which can be useful to guide therapeutic decision making between estrogen-targeting hormonal therapies versus chemotherapy. Additionally, FES-PET uptake at sites devoid of native estrogen receptor expression, such as bone, may be highly specific for breast cancer metastases.

According to the CEP-NCI [^{18}F]FES Investigator Drug Brochure Version 5 (2017), more than 20 fluorinated estrogen derivatives have been evaluated for imaging, and FES remains the most promising to date.

2.4 Overview of the FES/FDG SUVmax ratio

Several recent prior studies have shown potential predictive utility for progression free survival using the FES/FDG SUVmax ratio [48-50]. Based on a traditional understanding of physiology, indolent tumors would be predicted to have a lower glycolytic rate and therefore show low FDG uptake. Indolent tumors are also more likely to be well-differentiated and, for breast cancer, this would therefore suggest presence of functional ERs and high corresponding FES uptake. The most indolent, well-differentiated tumors may therefore show high FES uptake, low FDG uptake, and result in a high FES/FDG SUVmax ratio [28]. Additionally, as FES has been shown to be predictive of response to endocrine therapy, and FDG has been shown to predict

disease aggressiveness, it is possible that the combination of these factors will be predictive of progression free survival [28].

However, Liu, et al. [48] found the opposite to be true in a recent study on a population of ER+ metastatic breast cancer patients (not specific to ILC with 82.8% IDC and 11.4% ILC) undergoing treatment with fulvestrant. In this study, lesions were classified as heterogeneous (patient with multiple tumors demonstrating variable FES-PET/CT uptake) or, if homogeneous, as low or high FES/FDG ratios with a cut-point of 0.96 based on the median FES/FDG SUVmax ratio of the sample population. Neither FES SUVmax nor FDG SUVmax predicted progression free survival, but the FES/FDG ratio did separate patients based on median progression free survival for low FES/FDG (29.4 months) compared to high FES/FDG (14.7 months). The heterogeneous group had the lowest median progression free survival (5.5 months) suggesting that imaging evidence of estrogen receptor dysfunction in some lesions is associated with shorter median progression free survival. These three-way PET classifiers were shown to be independent, statistically significant prognostic factors for median progression free survival on fulvestrant therapy (p-value 0.006).

The results of the study by Liu, et al. [48] are unique from what the study authors hypothesized in that patients with low FES/FDG ratios showed longer median progression free survival when the opposite may be predicted based on FES uptake correlating with well-differentiated tumors and FDG uptake as a marker of metabolic aggressiveness. The study authors surmise that one reason may be 17B-estradiol activation of glucose uptake via ER-dependent PI3k/Akt activation in ER+ breast cancer cell lines [48].

2.5 Results from Study Pilot Phase:

The pilot phase of this study completed in 2021 by Dr. Covington and the Center for Quantitative Cancer Imaging at Huntsman Cancer Institute evaluated 17 patients (average age 58.4 years, all female sex) with ILC with FES-PET/CT and optional FDG-PET/CT imaging. The hypothesis of this trial was that FES-PET/CT would have superior detection of ILC compared to standard-of-care FDG-PET/CT, which, per a literature search, has a positive detection rate of 60%. This study showed positive (abnormal) FES uptake within 22/25 lesions (88%, p-value = 0.0024) and within 14/17 subjects (82.4%, p-value = 0.046).

Heterogeneous FES uptake defined as uptake in some but not all suspected sites of metastatic disease based on biopsy and imaging in patients with more than 1 lesion was identified in 2/10 lesions (2.5 to 55.6% exact 95% binomial confidence interval).

Discordant uptake was seen in 33/41 lesions and, for these lesions, there was significantly more FES+/FDG- than FES-/FDG+ cases (p = 0.016) using McNemar's test for correlated proportions. For cases with both FES and FDG imaging, Spearman correlation of lesion uptake based on maximal SUV uptake (SUVmax) showed a p-value of 0.00083 for biopsied lesions and a p-value of 0.0046 for all suspected abnormal lesions.

Interestingly, one patient with histologically proven ER- ILC from core needle biopsy was enrolled and did show abnormal FES uptake by qualitative and quantitative imaging assessment criteria. It is unknown whether this result is secondary to disease heterogeneity with only an ER- portion of a tumor being sampled, with separate ER+ components being unsampled, or if this reflects a false negative abnormal finding on FES-PET/CT imaging.

Of 17 cases of newly diagnosed ILC, 7 had histologically-proven nodal metastatic disease (42.8%) on core biopsy or sentinel lymph node biopsy and 3 of these (42.8%) were not detected on either standard of care ultrasound or MRI. Diagnostic performance of study imaging for detection of axillary nodal metastatic disease is shown in Table 2.5.1.

Table 2.5.1. Imaging performance for histologically proven axillary nodal metastatic disease.

	Sensitivity	Specificity	PPV	NPV	Accuracy	N=
Ultrasound	29%	90%	66.7%	64.3%	64.7%	17
MRI	57%	90%	80%	75%	76.5%	17
FDG-PET/CT	67%	100%	100%	80%	80%	14
FES-PET/CT	100%	100%	100%	100%	100%	17

Abbreviations: PPV: positive predictive value. NPV: Negative predictive value.

Six non-ILC malignant breast lesions were identified in our study population consisting of 2 IDC lesions and 4 lesions that were ductal carcinoma in situ (DCIS). Of these, FES-PET/CT identified 5 of 6, FDG-PET/CT identified all 6, contrast-enhanced breast MRI detected 3 of 6, ultrasound detected 1 of 4 (2 cases had no ultrasound imaging), and mammography detected 1 of 6. This suggests superiority of both FES- and FDG-PET/CT for identification of additional lesions compared to standard of care contrast-enhanced breast MRI, ultrasound, and mammography that will be further assessed in the present study.

We calculated the mean SUVmax FES/FDG ratio for our cohort of ILC patients was 1.2 compared to the mean ratio of 0.96 in the Liu, et al. study [48]. When assessing whether this ratio correlates with tumor grade, we found a p-value of 0.01 with R of -.6042 showing a moderate negative correlation between FES/FDG ratios and tumor grade, suggesting that higher grade tumors have lower FES/FDG ratios. When grade 1 or grade 2 disease as a function of the FES/FDG ratio being above or below the cut-point of 1.2, this cut-point was deemed significant with a p-value of 0.04.

When assessing whether lymph node metastatic disease varies as a function of FES/FDG ratios above or below a cut-point of 1.2 a p-value of 0.07 was found, and a

correlation coefficient R of 0.5004 with a p-value of 0.08, therefore suggesting a trend towards significance that will be further assessed in the present study.

2.6 Overview of MethylPatch PCR Analysis for Breast Cancer Evaluation

MethylPatch PCR analysis is a blood-based methylated circulating tumor DNA (ctDNA) assay that has been developed at the Huntsman Cancer Institute under the direction of K-T Varley, PhD. Many blood-based tumor marker tests have been developed to improve the detection of breast cancer [51, 52]. The most widely utilized is CA 15-3, but it is often detected in association with other non-malignant diseases and benign conditions, and has lower sensitivity for early stage disease and therefore cannot serve as a prognostic biomarker in clinical practice [51, 52]. There is a need for more sensitive and specific biomarkers for the detection of breast cancer. The discovery that tumor cells shed small amounts of DNA into the bloodstream, and that cancer-specific mutations can be detected by ultra-deep sequencing of DNA isolated from blood plasma has revolutionized the field of cancer diagnostics [53, 54]. Circulating tumor DNA (ctDNA) tests are a promising approach for detecting and monitoring breast cancer because a minimally invasive blood draw can be part of routine yearly wellness appointments [54].

The Methyl Patch PCR assay addresses several major challenges associated with using ctDNA for breast cancer screening. These include identifying markers present across patients and specific to cancer. Most ctDNA tests rely on the detection of mutations in targeted panels of cancer genes. Dr. Varley's lab analyzed tumor mutation data from The Cancer Genome Atlas (TCGA) and found that 18% (189/1066) of breast cancer tumors do not contain point mutations in 73 cancer genes covered by Guardant Health's field-leading ctDNA assay (Guardant 360) [55]. This analysis indicates that even if the mutation-based ctDNA test had perfect analytical sensitivity, it would be unable detect cancer in 18% of patients with breast cancers. Single base substitutions in TP53 are the most common somatic mutation in breast cancer, and often the only cancer gene mutation found in patient tumors, but TP53 mutations are also found in blood plasma of healthy individuals due to clonal hematopoiesis of indeterminate potential [56]. These age-related false-positive TP53 mutations in healthy individuals diminish the specificity of mutation-based ctDNA screening tests.

To address this challenge, Dr. Varley's lab studied a different marker of ctDNA: DNA methylation. Aberrant DNA methylation occurs early in tumor development at hundreds of specific genomic loci in each tumor. Many cancer genomics research groups, including Dr. Varley's, have identified hundreds of loci that exhibit cancer-specific methylation across cancer types [57-59]. While some patients' tumors contain few, or sometimes zero, mutations in targeted cancer gene panels, every tumor will have cancer-specific DNA methylation at the commonly methylated loci, ensuring specific detection of ctDNA in a larger portion of patients with cancer. The benefits of detecting methylated ctDNA were demonstrated in a recent ASCO presentation by the Circulating Cell-free Genome Atlas Study which reported that whole genome bisulfite

sequencing (a DNA methylation assay) had higher sensitivity than mutation-based assays [60].

Sensitive detection of early stage disease is also challenging using ctDNA assays. Early stage tumors produce lower amounts of cell-free DNA. In three recent studies that evaluated ctDNA mutation assays for cancer detection, the frequency of ctDNA detection in Stage 1 patients was approximately 50% [53, 61, 62]. This suggests that tumor-specific mutations were present at a concentration of 0.5 molecules per blood specimen. One way to increase the probability of observing rare molecules is to increase the amount of blood collected from each patient, which is often prohibited by Institutional Review Boards due to patient safety. Alternatively, measuring multiple mutations increases the probability of observing rare ctDNA. If you could detect 6 independent mutations in a patient, the probability of missing all 6 mutations due to undersampling is low, and 98% (1-0.5^6) of Stage I patients would have detectable ctDNA. Dr. Varley's lab analyzed tumor mutation data from The Cancer Genome Atlas (TCGA) and found that less than 5% of breast cancers had 6 or more mutations in cancer genes. This result indicates that cancer gene panel assays are unlikely to be sensitive enough for detection of rare ctDNA molecules in early stage disease. To increase the sensitivity and specificity of ctDNA mutation assays for detecting breast cancer, several recent studies have performed exome sequencing on patients' tumors and developed custom assays to detect multiple mutations specific to each patient's tumor. In two recent studies using this approach, ctDNA was detected a median of 8.9 and 10.7 months prior to clinically detectable disease recurrence, with sensitivities of 89% and 96% [63, 64]. The success of these studies demonstrates that ctDNA assays can provide sensitive detection of breast cancer. Unfortunately, this strategy relies on sequencing a patient's tumor to identify their unique mutation profile for assay development, and thus cannot be used for initial diagnosis. A recent study that included both a 'cancer gene' mutation panel and protein markers showed improved specificity for initial detection of breast cancer (99%), but the sensitivity for detecting breast cancer was only 33% [61]. Additionally, broader implementation of this type of multi-analyte test will inherently face challenges related to robustness and feasibility. Together these studies suggest that multiplexed biomarker tests are a promising direction to pursue, but a more universal, single-analyte approach is needed.

An individual patient's tumor contains many more methylation defects than mutations [65]. While it is unlikely that a patient's tumor will contain 6 point mutations in a targeted cancer gene panel, the presence of 6 cancer-specific methylation events in a single patient is expected, because hundreds of loci are concurrently hyper-methylated in each tumor. A multiplexed ctDNA test designed to detect cancer-specific hypermethylation at many loci increases the probability of observing rare ctDNA molecules in the blood specimen. This enables robust and sensitive detection of early stage tumors that release smaller quantities of DNA into the bloodstream.

An additional drawback of using ctDNA mutation assays for early diagnosis is that many different tumor types contain the same mutations. The genes that are most frequently mutated in one cancer type (>20% of tumors) are also frequently mutated in other

cancer types (Figure 2.6.1A). If a ctDNA test detected a TP53 mutation during routine screening, it could indicate that the individual had cancer, but because TP53 mutations occur in all tumor types, it doesn't predict the patient's tumor type or location. This would lead to expensive follow-up imaging of multiple organs to identify the primary source of malignancy and determine which oncology subspecialist should manage the patient's care. In contrast, different cancer types have distinct methylation profiles [66-68] (Figure 2.6.1B). While most mutations are not unique to specific cancer types, if a breast cancer-specific DNA methylation profile is detected in the blood, it indicates a specific diagnosis of breast cancer leading to a more straightforward diagnostic breast imaging workup and referral to the appropriate breast oncology specialist. This study will utilize a new assay, called MethylPatch PCR, which is designed to provide deep targeted sequencing of 54 regions of the genome that exhibit DNA methylation specifically in breast cancer. The multiplexed quantification of methylated ctDNA from many loci is a promising approach to achieve a more specific, sensitive and robust blood-based tumor marker of breast cancer.

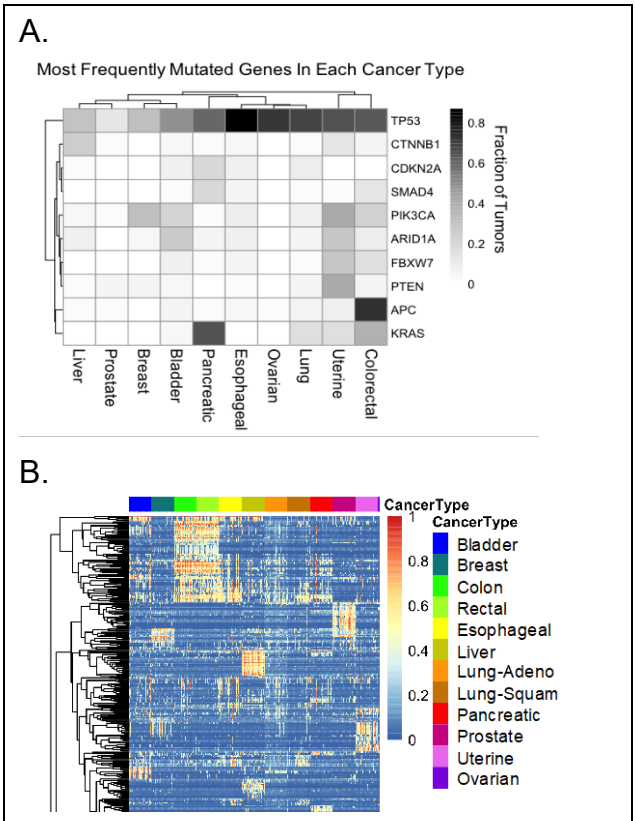


Figure 2.6.1. A. The most common mutations in each cancer are found in other cancer types. B. Heatmap of genomic loci that exhibit cancer-type specific hypermethylation in TCGA data.

The MethylPatch PCR assay is designed to provide deep targeted sequencing of 54 regions of the genome that exhibit DNA methylation in breast tumors. To determine if this approach could be used to detect methylated ctDNA, Dr. Varley's lab performed the MethylPatch PCR assay on DNA isolated from blood plasma collected from 15 breast cancer patients in the 2-month interval between diagnosis and surgical resection of their tumors. They also performed the assay on DNA isolated from blood plasma collected from 20 healthy donors. They calculated the percentage of methylated UMIs (molecules) observed for each target region in each sample. They then calculated normalized percent of methylated values for each target region in each sample by fitting the values to a standard curve using coefficients from a linear regression of the observed and expected values in a titration (Figure 2.6.2). In the healthy donor plasma samples, they observed fewer target regions with percent methylated values above 0.15 (LOD), and the mean of values in each of the healthy donor samples was lower than 0.15 (Figure 7). The breast cancer patient plasma

samples all had a larger number of target regions with percent methylated values above 0.15 (LOD), and all produced mean normalized percent methylated values greater than 0.15. The presence of higher quantities of methylated DNA in all of the breast cancer samples compared to all of the healthy donor samples confirms that our approach can detect low levels of methylated ctDNA in blood plasma samples from patients with breast cancer. Notably, four of the breast cancer patients were diagnosed with Stage I disease, demonstrating the ability to detect methylated ctDNA in patients with early stage disease and small tumors (Figure 2.6.3). These results confirm that the MethylPatch PCR assay can detect and quantify methylated ctDNA specifically in breast cancer patients, and demonstrates that the highly multiplexed nature of this assay (54 markers) provides more opportunities to sensitively and robustly detect rare ctDNA in each patient.

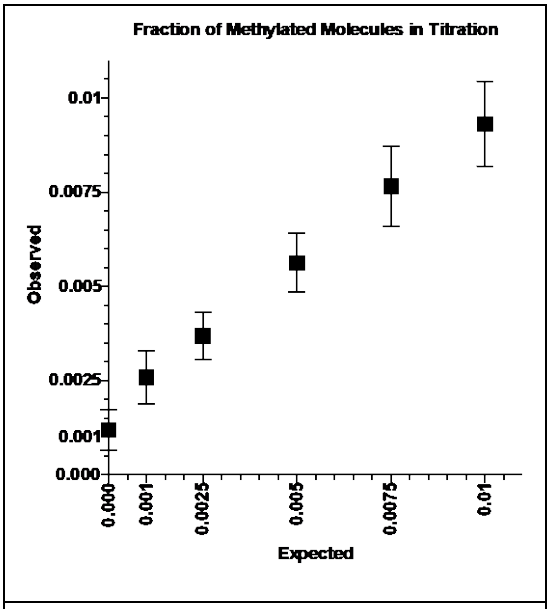


Figure 2.6.2. MethylPatch PCR can accurately quantify low levels of methylated DNA titrated into cell-free DNA from healthy donors. Mean and 95% confidence intervals are depicted.

Preliminary data from Dr. Varley's lab has also identified genes in triple negative breast cancer that, when highly methylated, predict an early clinical relapse. Additionally, early data suggests that if the methylation pattern identified in metastatic disease is seen when only primary breast malignancy but no metastatic disease is evident, this is also predictive of an early clinical relapse. Such cancer-specific DNA methylation changes on ctDNA in blood plasma may predate clinical relapse by approximately 10 months. Ideally such markers should be monitored longitudinally throughout treatment and remission, and this study will provide this necessary and important information for patients with ILC.

Pertinent to the current study, the number of circulating tumor cells are higher in ILC compared to IDC but the prognostic nature of having a higher circulating tumor cell burden was weaker compared to IDC suggesting that the circulating tumor cells in ILC are more dormant [69]. Additionally, there is a current challenge to differentiate between ILC tumors that have a worse prognosis given the near-uniformity in terms of ER and PR positivity, HER2 negative status, proliferation index, and grade [70]. This study will evaluate, in a longitudinal manner, whether MethylPatch PCR ctDNA analysis provides additional prognostic value beyond these standard-of-care metrics.

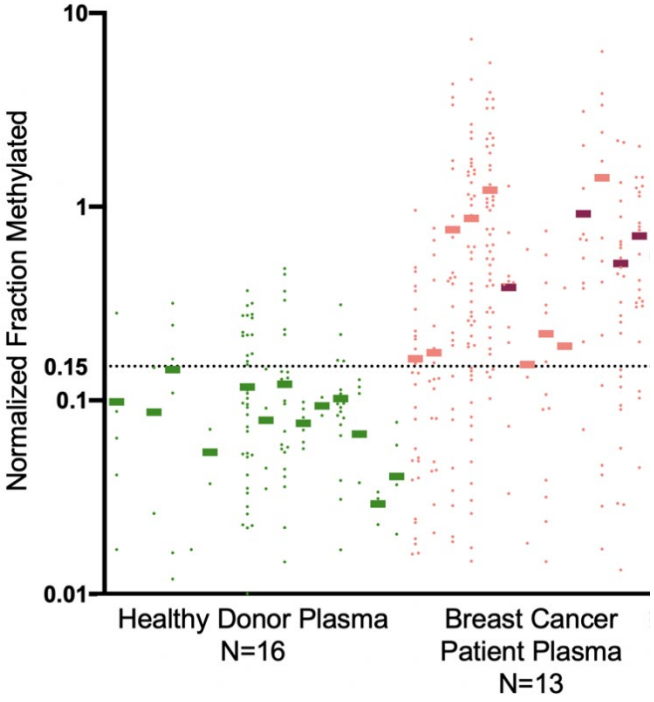


Figure 2.6.3. Breast cancer patient plasma samples (N=15) contained methylated ctDNA from more target genomic regions (dots), higher percentage of methylated molecules from each region, and higher mean percentages of methylated molecules across regions (rectangles) compared to health donor plasma samples (green, N=20). Stage I breast cancer patients are indicated by light pink dots.

3. PHARMACOLOGY, SAFETY and RADIATION DOSIMETRY of [^{18}F]FES

3.1. Pharmacology and Safety

3.1.1. Pharmacology of [^{18}F]FES in Humans

The information provided herein is abstracted from the CEP-NCI [^{18}F]FES Investigator Drug Brochure Version 5 (2017).

The pharmacology of FES is best understood by analogy to estradiol, a naturally occurring product synthesized in the ovary in pre-menopausal women and by

conversion from adrenal steroids, largely through the action of aromatase enzymes. Pre-menopausal levels of estradiol vary widely during the menstrual cycle, reaching levels as high as 500 pg/ml (1.7 nM) mid-cycle. In post-menopausal women and in men, levels are generally less than 30 pg/ml (0.1 nM). Because FES-PET is not intended for therapeutic effect and is not used in sufficient concentration to elucidate a physiologic effect, mechanisms of action beyond metabolism and binding to ER have not been studied.

Typical blood FES concentration after a 6 mCi injection is 1 μCi/ml (< 3 pmoles/ml) peak and by 60 minutes after injection is less than 150 fmoles/ml. For the limiting-case specific activity this corresponds to a peak blood FES level of 833 pg/ml and 42 pg/ml at one hour, comparable to pre-menopausal mid-cycle estradiol levels of 62 to 534 pg/ml and post-menopausal levels of 20 to 88 pg/ml. Males have levels similar to post-menopausal women. Thus the short-term exposure to estrogen from an FES injection as part of an FES PET study transiently yields physiologic levels of the estrogenic steroid, decreasing to sub-physiologic levels after 60 minutes.

The metabolism of estradiol has been well characterized. It occurs largely in the liver. The formation in the liver of sulfate conjugates at hydroxyl sites is an important route of metabolism. These conjugates are secreted into bile and have efficient enterohepatic circulation that serves as a reservoir for regulating estrogen levels. Glucuronides are also formed in the liver, to a lesser extent than sulfates, and their primary route of elimination is the urine.

3.1.2. Toxicity of [^{18}F]FES in Humans

The information provided here is abstracted from the [^{18}F]FES Investigator Drug Brochure, Version 5 (2017), with minor changes to reflect our local manufacturing process. The [^{18}F]FES is a sterile, IV injectable solution in sodium chloride buffered by sodium ascorbate. The injected dose of [^{18}F]FES is 6 mCi (185 MBq), with an allowable range of 3 to 6 mCi and a specific activity greater than 170 Ci/mmol at the time of injection. FES is the only active ingredient. There is no evidence that nonradioactive and radioactive FES molecules display different biochemical behavior. Approximately 1500 patients are known to have received this drug, based upon published reports, without significant adverse effects.

[^{18}F]FES is purified by HPLCand is formulated in a solution 0.9% (v/v) sodium phosphates for injection (USP), less than 10% ethanol (v/v) in normal saline mL with a maximum injection volume of 5 ml.. The concentration of ethanol in the final injectate is less than 32 mg/ml, or a maximum of 0.505 ml of ethanol. This is less than one-third of the amount of ethanol in one alcoholic drink and <0.05 ml/kg (< 0.04g/kg) for a standard 56.8-kg woman. In RTECS, the LD_{Lo} is given as 1.4 g/kg orally for producing sleep, headache, nausea, and vomiting. Ethanol has also been administered intravenously to women experiencing premature labor (8 g/kg) without producing any lasting side effects. Based upon these reports and experience with over 100 patients over the past decade

receiving this amount of ethanol in injectates, we conclude that ethanol will not pose any danger of toxicity in this study.

The other components of the final product solution; sterile water for injection, saline and sodium phosphates are all USP grade. These are all nontoxic for USP grade injectables at the concentrations that will be used. The final product is at pH 6.5 and the final injection volume is ≤ 5 ml.

The potential contaminants in the final [^{18}F]FES drug product are: acetone, acetonitrile, Kryptofix®, other reaction products. Residual solvents in the final product are limited to 410 ppm of acetonitrile. Acetonitrile is used to dissolve the Kryptofix® [2.2.2] and is the solvent for the reaction. The permissible level of acetonitrile in the final product is ≤ 410 ppm, the USP permissible level of acetonitrile in 2-[^{18}F]FDG. All of the residual solvent levels met our acceptance criteria in our initial three qualification syntheses.

The toxicity for Kryptofix® has not been reported (RTECS Number Kryptofix® 222 MP4750000). The MSDS for Kryptofix® lists the LD_{50} for intravenous administration in rats and mice as 35 and 32 mg/kg respectively. In light of these reported toxicities, the FDA has proposed a maximum permissible level of 50 µg/ml of Kryptofix® in 2-[^{18}F]FDG, as has the USP[i]; therefore, this maximum permissible level will also apply to the [^{18}F]FES final product. All of the Kryptofix levels were < 50 µg/ml in our initial qualification syntheses (n=3).

Although a relatively pure [^{18}F]FES product is obtained, trace amounts of other reaction products may be found in the final product. For this reason, an upper limit of 5 µg per dose has been set for the total mass of any other materials in the final injectate. The 5 µg is determined by assuming that the UV absorbing compounds at 280 nm have the same molar extinction coefficient as FES.

Pregnant or nursing women: Reproductive toxicology studies have not been performed with [^{18}F]FES. However, since [^{18}F]FES is a radiopharmaceutical, it is assumed that fetal toxicity or embryo fetal toxicity may result if it is administered to a pregnant woman. Therefore, [^{18}F]FES injection is not suitable for administration to pregnant or nursing women.

Genotoxic and mutagenic potential: Carcinogenesis does not appear to be a risk of administration of [^{18}F]FES as described in the Investigator's Brochure based upon a large number of animal toxicity studies. Although estrogens, including the natural hormones estradiol and estrone, are carcinogenic in laboratory animals, 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. [^{18}F]FES is labeled with fluorine at the 16 position of the estradiol. Administration of [^{18}F]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.

Findings in safety pharmacology and toxicology studies: Nonclinical toxicology and safety pharmacology studies with [^{18}F]FES have not identified any specific target organs or adverse effects on nervous, respiratory, or cardiovascular systems. As such, no potential [^{18}F]FES injection-related safety risks for humans have been identified on the basis of the nonclinical data.

Expected risks to subjects participating in clinical studies: No serious adverse reactions have been reported from any published studies. [^{18}F]FES could potentially exert toxic effects through 1 of 3 mechanisms: (1) radiation exposure to tissues from the radioactive label, (2) physiologic actions mediated through the ER, and (3) direct toxic or mutagenic effects of FES or metabolites. Radiation exposure from [^{18}F]FES at injected activities used in PET (6 mCi, typical) is low, and is comparable to other nuclear medicine procedures. With respect to the other two mechanisms of toxicity, FES injected as a bolus for PET imaging (current mass dose limit of 5 micrograms) transiently reaches physiologic concentrations, but returns to sub-physiologic levels within an hour after injection. As such, toxic effects due to actions mediated through the ER and directly toxic effects of metabolites will be far less than those of natural ER ligands. All of the evidence supports the safety of [^{18}F]FES-PET imaging. Intravenous injection and the use of an intravenous cannula are known to carry a small risk of infection and hematoma. The exposure to radiation will not exceed that which is considered acceptable in accordance with appropriate guidelines.

Reports of intravenous administration of estrogens are rare; it is used in this form largely in the setting of acute dysfunctional uterine bleeding. Two studies have documented acute toxicities resulting from intravenous bolus doses. White and colleagues studied pharmacokinetics and tolerability of 17β-estradiol in eight postmenopausal women [71]. Estradiol was administered in doses of 25, 50, 100, or 200 μg peripherally over a five second period. Peak serum levels were not reported; however, the authors did document approximate dosage proportionality with respect to serum area under the curve (AUC). An adverse event was reported in only one patient who experienced mild discomfort at the injection site immediately following her dose. This reaction lasted 3 – 4 seconds and did not recur.

Intravenous administration of Premarin® 25 mg versus placebo in 34 patients with dysfunctional uterine bleeding resulted in mild adverse reactions reported for seven of 18 treated patients (39%) versus two of 16 (13%) in the placebo group [72]. Adverse effects in the Premarin® treated patients included flushing, euphoria, dizziness, drowsiness, and taste disturbances. The mean changes from baseline following injection of Premarin® versus placebo for blood pressure, pulse and respiratory rate were not statistically significant. In another study, intravenous estradiol combined with oral estradiol was given in post-menopausal women with recurrent ischemia and history of unstable angina [73]. This study reported a low incidence of headache, edema, vaginal bleeding, and a 23% incidence of breast tenderness and mood changes. Results of these studies are shown in Table 3.1.

Table 3.1. Adverse events from selected trials of intravenous estradiol

Number of Patients	Drug	Dose	Duration	Adverse Events	Estradiol Blood Levels	Source
8	IV estradiol	25 □g 50 □g 150 □g 300 □g	Single injection	No reported AE	Baseline = 42.2 ng/L 60 mins post inj = 689.6 ng/L	Rosano 1993[74]
18	IV Premarin ® (Conjugated Equine Estradiol)	25 mg	Dual injection	Flushing, drowsiness, euphoria, dizziness, nausea	Not reported	Devore 1982[72]
100	IV estradiol + Oral estradiol	1.25 mg + 1.25 mg/QD	Single injection + 21 days of oral E	○ flushing 7% ○ headache 11% ○ edema 11% ○ Vaginal bleeding 10% ○ breast tenderness 23% ○ mood changes 5%	Not reported	Schulman[73]

The Rosano et al study is most relevant to the use of FES-PET[74]. For this study up to 300 µg of estradiol was administered intravenously. Blood levels of estradiol were up to 690 ng/L (pg/ml) at 60 minutes post injection, and there were no reported adverse events. For FES used for PET, the typical dose is 1.5 µg or less, with a maximum dose of 5 µg, and for the dose limit injection the blood level at 60 minutes should be 42 pg/ml. PET studies also indicate that the tissue distribution and blood clearance of [^{18}F]FES is similar to IV estradiol.

With the exception of discomfort at the injection site, adverse events to FES IV injection at doses used for PET/CT have not been reported [75].

3.2. Human Radiation Dosimetry

3.2.1. Human Radiation Dosimetry of [^{18}F]Fluoroestradiol

The amount of injected [^{18}F]FES activity used in this protocol will be 222 MBq (6 mCi). The dosimetry estimates based on 49 patients was previously published and is used to estimate radiation dose and risks in this protocol [76]. The greatest organ absorbed doses for a 222 MBq (6 mCi) injection of [^{18}F]Fluoroestradiol are the liver (28 mGy) and gallbladder (23 mGy), while the effective dose is 4.9 mSv (Table 3.3).

3.2.2. Human Radiation Dosimetry of [^{18}F]Fluorodeoxyglucose

The amount of injected [^{18}F]FDG activity used in this protocol will be 555 MBq (15.0 mCi). This is the typical activity used for a clinical whole-body PET study. The radiation dosimetry estimates for [^{18}F]FDG are based on Publication 106 issued by The International Commission on Radiation Protection (ICRP) [International Commission on Radiological Protection. Radiation dose to patients from radiopharmaceuticals. Addendum 4 to ICRP Publication 53. ICRP Publication 106. Feb 24, 2014.]. The greatest organ absorbed doses for a 555 MBq (15 mCi) injection of [^{18}F]FDG are the bladder (72 mGy) and the heart (37 mGy), while the effective dose is 10.6 mSv (Table 3.4).

3.2.2 Human Radiation Dosimetry for CT exams performed on the PET/CT Scanner

The study will be performed on the research GE Discovery 710 PET/CT scanner in the Molecular Imaging Suite at HCI. Each PET imaging study will require a helical CT scan from the top of the head through the knees with near diagnostic technique for both clinical interpretation and attenuation correction. The CT acquisition parameters for large patients receiving the greatest exposure will be 140 kVp, 0.5s rotation speed, 250 mA tube current, 64 x 0.625 mm collimation, and a pitch of 1.35 for the helical scan. Note that most patients will utilize 120 kVp but dosimetry estimates are based on larger patients at 140 kVp. Automatic tube current modulation is used for all CT exams but for the purpose of estimating maximum risk, a maximum fixed tube current of 500 mA is used for all calculations in order to provide a conservative estimate of maximum risk. In order to estimate the absorbed doses of individual organs and the resulting effective dose, the ImPACT Scan CT Patient Dosimetry Calculator (Version 1.0.4) was used with the specific acquisition parameters used in this protocol and the NRPB monte carlo dose data sets for the GE Lightspeed VCT scanner produced in report SR250 dosimetry tables [3.) ImPACT, Imaging Performance Assessment of Computed Tomography Scanners (online). 2006, St. George's Hospital, ImPACT group: Tooting, London. http://www.impactscan.org/index.htm. 4.) Shrimpton, P.C., et al., Survey of CT Practice in the UK. Part 2: Dosimetric Aspects, NRBP-R249. 1991: London. 5.) Jones DG, Shrimpton PC. Survey of CT practice in the IK. Part 3: Normalized organ doses calculated using Monte Carlo techniques. Chilton, NRPB-SR250. 1991, (London, HMSO).]. A correction factor has been applied to account for the difference in the CT Dose index (CTDIvol) derived from ImPACT Calculator and that reported on the GE scanner. Note that the topogram contributes a negligible radiation exposure to the helical CT exam and was not included in the dose estimates. The greatest organ absorbed doses for a single helical body CT scan using the aforementioned acquisition parameters are bone surfaces (28 mGy) and thyroid (27 mGy), while the effective dose is 17 mSv (Table 3.3).

3.2.3. Cumulative Radiation Dosimetry for ^{18}F]Fluoroestradiol-PET/CT only

The cumulative radiation dose to a research subject participating in this clinical trial and receiving only the mandatory [^{18}F]Fluoroestradiol-PET/CT has been compiled from the dosimetry estimates for a single [^{18}F]Fluoroestradiol-PET study and the corresponding whole body CT scan that will be performed as part of the PET/CT procedure. For research subjects participating in the clinical trial and receiving only the single [^{18}F]Fluoroestradiol-PET/CT study, the greatest cumulative organ absorbed doses for the study are the liver (44 mGy), and gallbladder (39 mGy) while the effective dose is 22 mSv (Table 3.3).

3.2.4. Cumulative Radiation Dosimetry for [^{18}F]Fluoroestradiol-PET/CT and [^{18}F]FDG-PET/CT

For Pilot Phase Completed in 2021:

The cumulative radiation dose to a research subject participating in this clinical trial and receiving both the mandatory [^{18}F]Fluoroestradiol-PET/CT and the optional [^{18}F]FDG-PET/CT has been compiled from the dosimetry estimates for a single [^{18}F]Fluoroestradiol PET study (Table 3.3), a single [^{18}F]FDG-PET study, and the 2 corresponding whole body CT scans that will be performed as part of the PET/CT procedures (Table 3.4). For research subjects participating in the clinical trial and receiving both the single [^{18}F]Fluoroestradiol PET/CT study and the single [^{18}F]FDG-PET/CT study, the greatest cumulative organ absorbed doses for the study are the bladder (118 mGy, heart (78 mGy), and liver (72 mGy), while the effective dose is 49 mSv (Table 3.4).

Table 3.3. Radiation dosimetry table for [^{18}F]FES-PET/CT

IRB #128055	FES-PET	Helical Body CT	PET/CT Total
Activity (mCi)	6	-	-
Number of Scans	1	1	-
Organ		Single Scan Absorbed dose (mGy)	Protocol Absorbed dose (mGy)
Adrenals	5.11	15.43	20.54
Bladder	11.10	17.77	28.87
Bone surfaces	3.11	28.05	31.16
Brain	2.22	18.23	20.45
Breasts	2.00	14.96	16.96
Gallbladder	22.64	16.36	39.01
Stomach	3.11	16.83	19.94
Small intestine	5.99	15.43	21.42
Colon	4.35	15.43	19.78
Heart	5.77	17.77	23.54
Kidneys	7.77	17.77	25.54
Liver	27.97	16.36	44.34
Lungs	3.77	18.70	22.48
Muscles	4.66	13.56	18.22
Oesophagus	3.11	20.11	23.21
Ovaries	4.00	14.96	18.96
Pancreas	5.11	14.96	20.07
Red marrow	2.89	13.09	15.98
Skin	1.11	12.62	13.73
Spleen	3.33	15.90	19.23
Testes	2.66	19.17	21.83
Thymus	3.11	20.11	23.21
Thyroid	2.66	26.65	29.32
Uterus	8.66	15.43	24.09
Eye Lens	2.00	21.51	23.51
Effective dose (mSv)	4.88	16.83	21.72

Table 3.4. Radiation dosimetry table for [^{18}F]FES-PET/CT + [^{18}F]FDG-PET/CT

IRB #128055	FES-PET	FDG-PET	Helical Body CT	PET/CT Total
Activity (mCi)	6	15	-	-
Number of Scans	1	1	2	-
Organ		Single Scan Absorbed dose (mGy)	Single Scan Absorbed dose (mGy)	Protocol Absorbed dose (mGy)
Adrenals	5.11	6.66	15.43	42.63
Bladder	11.10	72.15	17.77	118.78
Bone surfaces	3.11	6.11	28.05	65.32
Brain	2.22	21.09	18.23	59.78
Breasts	2.00	4.88	14.96	36.81
Gallbladder	22.64	7.22	16.36	62.59
Stomach	3.11	6.11	16.83	42.88
Small intestine	5.99	6.66	15.43	43.51
Colon	4.35	7.22	15.43	42.42
Heart	5.77	37.19	17.77	78.49
Kidneys	7.77	9.44	17.77	52.74
Liver	27.97	11.66	16.36	72.36
Lungs	3.77	11.10	18.70	52.28
Muscles	4.66	5.55	13.56	37.33
Oesophagus	3.11	6.66	20.11	49.98
Ovaries	4.00	7.77	14.96	41.69
Pancreas	5.11	7.22	14.96	42.24
Red marrow	2.89	6.11	13.09	35.17
Skin	1.11	4.33	12.62	30.69
Spleen	3.33	6.11	15.90	41.23
Testes	2.66	6.11	19.17	47.11
Thymus	3.11	6.66	20.11	49.98
Thyroid	2.66	5.55	26.65	61.52
Uterus	8.66	9.99	15.43	49.51
Eye Lens	2.00	21.09	21.51	66.10
Effective dose (mSv)	4.88	10.55	16.83	49.09

For Expansion Phase Added in March 2022 Amendment:

3.2.5. Cumulative Radiation Dosimetry for [^{18}F]Fluoroestradiol-PET/CT and [^{18}F]FDG-PET/CT

The cumulative radiation dose to a research subject participating in this clinical trial and receiving both the mandatory [^{18}F]Fluoroestradiol-PET/CT and the optional FDG-PET/CT has been compiled from the dosimetry estimates for a single [^{18}F]Fluoroestradiol-PET study, a single FDG-PET study, and the 2 corresponding wholebody CT scans (1 moderate dose and 1 diagnostic dose) that will be performed as part of the PET/CT procedures. For research subjects participating in the clinical trial and receiving both the single [^{18}F]Fluoroestradiol PET/CT study and optional FDG-PET/CT study, the greatest cumulative organ absorbed doses for the study are the

bladder (136 mGy), heart (96 mGy), and bone (94 mGy), while the effective dose is 66 mSv (Table 3.5)

Table 3.5. Radiation dosimetry table for [^{18}F]FES-PET/CT + [^{18}F]FDG-PET/CT

IRB #128055	FES-PET	FDG-PET	Moderate Dose CT	Full Dose CT	PET/CT Total
Activity (mCi)	6	15	-	-	-
Number of Scans	1	1	1	1	-
Organ	Single Scan Absorbed dose (mGy)	Single Scan Absorbed dose (mGy)	Single Scan Absorbed dose (mGy)	Single Scan Absorbed dose (mGy)	Protocol Absorbed dose (mGy)
Adrenals	5.11	6.66	15.43	30.86	58.05
Bladder	11.10	72.15	17.77	35.53	136.55
Bone surfaces	3.11	6.11	28.05	56.11	93.37
Brain	2.22	21.09	18.23	36.47	78.01
Breasts	2.00	4.88	14.96	29.92	51.77
Gallbladder	22.64	7.22	16.36	32.73	78.95
Stomach	3.11	6.11	16.83	33.66	59.71
Small intestine	5.99	6.66	15.43	30.86	58.94
Colon	4.35	7.22	15.43	30.86	57.85
Heart	5.77	37.19	17.77	35.53	96.26
Kidneys	7.77	9.44	17.77	35.53	70.51
Liver	27.97	11.66	16.36	32.73	88.72
Lungs	3.77	11.10	18.70	37.40	70.98
Muscles	4.66	5.55	13.56	27.12	50.89
Oesophagus	3.11	6.66	20.11	40.21	70.08
Ovaries	4.00	7.77	14.96	29.92	56.65
Pancreas	5.11	7.22	14.96	29.92	57.21
Red marrow	2.89	6.11	13.09	26.18	48.27
Skin	1.11	4.33	12.62	25.25	43.31
Spleen	3.33	6.11	15.90	31.79	57.13
Testes	2.66	6.11	19.17	38.34	66.28
Thymus	3.11	6.66	20.11	40.21	70.08
Thyroid	2.66	5.55	26.65	53.30	88.17
Uterus	8.66	9.99	15.43	30.86	64.94
Eye Lens	2.00	21.09	21.51	43.02	87.61
Effective dose (mSv)	4.88	10.55	16.83	33.66	65.93

3.2.6. Two [18F]FES-PET/CT at baseline and 2-4 weeks following therapy

The cumulative radiation dose to a research subject participating in this clinical trial and receiving two [^{18}F]Fluoroestradiol-PET/CT scans has been compiled from the dosimetry estimates for two [^{18}F]Fluoroestradiol-PET study, and the 2 corresponding wholebody moderate dose CT scans that will be performed as part of the PET/CT procedures. For research subjects participating in the clinical trial and receiving both the baseline and post-therapy [^{18}F]Fluoroestradiol PET/CT studies, the greatest cumulative organ absorbed doses for the study are the liver (89 mGy), gall bladder (78 mGy), and bone (62 mGy), while the effective dose is 43 mSv (Table 3.6).

Table 3.6. Radiation dosimetry table for 2 FES-PET/CT.

IRB #128055	FES-PET	Moderate Dose CT	PET/CT Total
Activity (mCi)	6	-	-
Number of Scans	2	2	-
Organ	Single Scan Absorbed dose (mGy)	Single Scan Absorbed dose (mGy)	Protocol Absorbed dose (mGy)
Adrenals	5.11	15.43	41.07
Bladder	11.10	17.77	57.73
Bone surfaces	3.11	28.05	62.32
Brain	2.22	18.23	40.91
Breasts	2.00	14.96	33.92
Gallbladder	22.64	16.36	78.02
Stomach	3.11	16.83	39.88
Small intestine	5.99	15.43	42.85
Colon	4.35	15.43	39.56
Heart	5.77	17.77	47.08
Kidneys	7.77	17.77	51.07
Liver	27.97	16.36	88.67
Lungs	3.77	18.70	44.95
Muscles	4.66	13.56	36.44
Oesophagus	3.11	20.11	46.43
Ovaries	4.00	14.96	37.92
Pancreas	5.11	14.96	40.14
Red marrow	2.89	13.09	31.96
Skin	1.11	12.62	27.47
Spleen	3.33	15.90	38.45
Testes	2.66	19.17	43.67
Thymus	3.11	20.11	46.43
Thyroid	2.66	26.65	58.63
Uterus	8.66	15.43	48.18
Eye Lens	2.00	21.51	47.01
Effective dose (mSv)	4.88	16.83	43.43

3.2.7. [18F]FES-PET/CT and [18F]FDG-PET/CT at baseline and [18F]FES-PET/CT 2-4 weeks following therapy

The cumulative radiation dose to a research subject participating in this clinical trial and receiving two [^{18}F]Fluoroestradiol-PET/CT and the optional FDG-PET/CT has been compiled from the dosimetry estimates for a two [^{18}F]Fluoroestradiol-PET studies, a single FDG-PET study, and the 3 corresponding wholebody CT scans (2 moderate dose and 1 diagnostic dose) that will be performed as part of the PET/CT procedures. For research subjects participating in the clinical trial and receiving both the two[^{18}F]Fluoroestradiol PET/CT study and the single FDG-PET/CT study, the greatest cumulative organ absorbed doses for the study are the bladder (165 mGy), liver (133 mGy), and bone (125 mGy), while the effective dose is 88 mSv (Table 3.7).

Table 3.7. Radiation dosimetry table for 2 FES-PET/CT and 1 FDG-PET/CT.

IRB #128055	FES-PET	FDG-PET	Moderate Dose CT	Full Dose CT	PET/CT Total
Activity (mCi)	6	15	-	-	-
Number of Scans	2	1	2	1	-
Organ	Single Scan Absorbed dose (mGy)	Single Scan Absorbed dose (mGy)	Single Scan Absorbed dose (mGy)	Single Scan Absorbed dose (mGy)	Protocol Absorbed dose (mGy)
Adrenals	5.11	6.66	15.43	30.86	78.59
Bladder	11.10	72.15	17.77	35.53	165.42
Bone surfaces	3.11	6.11	28.05	56.11	124.54
Brain	2.22	21.09	18.23	36.47	98.47
Breasts	2.00	4.88	14.96	29.92	68.73
Gallbladder	22.64	7.22	16.36	32.73	117.96
Stomach	3.11	6.11	16.83	33.66	79.65
Small intestine	5.99	6.66	15.43	30.86	80.37
Colon	4.35	7.22	15.43	30.86	77.63
Heart	5.77	37.19	17.77	35.53	119.80
Kidneys	7.77	9.44	17.77	35.53	96.04
Liver	27.97	11.66	16.36	32.73	133.06
Lungs	3.77	11.10	18.70	37.40	93.46
Muscles	4.66	5.55	13.56	27.12	69.11
Oesophagus	3.11	6.66	20.11	40.21	93.30
Ovaries	4.00	7.77	14.96	29.92	75.61
Pancreas	5.11	7.22	14.96	29.92	77.27
Red marrow	2.89	6.11	13.09	26.18	64.24
Skin	1.11	4.33	12.62	25.25	57.05
Spleen	3.33	6.11	15.90	31.79	76.35
Testes	2.66	6.11	19.17	38.34	88.11
Thymus	3.11	6.66	20.11	40.21	93.30
Thyroid	2.66	5.55	26.65	53.30	117.48
Uterus	8.66	9.99	15.43	30.86	89.02
Eye Lens	2.00	21.09	21.51	43.02	111.12
Effective dose (mSv)	4.88	10.55	16.83	33.66	87.64

4. TRIAL DESIGN

4.1. Patient Eligibility

For Pilot Phase Completed in 2021

Study patients: Adult patients, (n = 24 tumors).

The study duration is expected to be 1-2 years.

For Expansion Phase Added in March 2022 Amendment:

Additional patients to be enrolled per March 2022 study amendment:

Study patients: Adult patients, (n = 55) to achieve at least 38 evaluable patients for the primary endpoint and up to 15 evaluable patients for exploratory objectives.

The study duration is expected to be 5 years.

4.2. Inclusion Criteria

For Pilot Phase Completed in 2021:

- Adults aged 18 years or greater
- All patients or legal guardians are willing and able to sign a written informed consent and HIPAA authorization in accordance with local and institutional guidelines.
- Histologically confirmed invasive lobular carcinoma within the past 12 weeks confirmed from biopsy of primary tumor or metastasis.
- Patient is willing to have their clinical records reviewed for at least 24 months after enrollment.

For Expansion Phase Added in March 2022 Amendment:

- Adults aged 18 years or greater
- All patients or legal guardians are willing and able to sign a written informed consent and HIPAA authorization in accordance with local and institutional guidelines.
- Patient must qualify for **one** of the following:
    - Primary endpoint analysis/Primary Arm:
        - Histologically confirmed ER+ invasive lobular carcinoma within the past 16 weeks confirmed from biopsy of primary tumor or metastasis (n=40).
    - Exploratory Arm 1:
        - Histologically confirmed ER+ invasive lobular carcinoma at any time in the past, confirmed from biopsy of primary tumor or metastasis, with confirmed or imaging suspected metastatic disease, currently on antihormonal therapy or chemotherapy (n=10).
    - Exploratory Arm 2:
        - Histologically confirmed ER- invasive lobular carcinoma (at any point) at any site with biopsy-proven or imaging suspected metastatic ILC (n=5).
- Patient is willing to have their clinical records reviewed, and be contacted by phone during follow-up intervals specified, for approximately 60 months after enrollment.
- Patient is willing to provide baseline blood specimens for ctDNA analysis.

4.3. Exclusion Criteria

For Pilot Phase Completed in 2021:

- Patients with known allergic or hypersensitivity reactions to previously administered radiopharmaceuticals. Patients with significant drug or other allergies or autoimmune diseases may be enrolled at the Investigator's discretion.
- Patients who require monitored anesthesia for PET/CT scanning.
- Patients who are too claustrophobic to undergo PET/CT scanning.
- Pregnancy or current breast feeding.
- Any patient that is medically unstable defined as a patient requiring inpatient hospitalization or needing evaluation at an acute care or urgent care facility at time of imaging.
- Patients undergoing treatment with estrogen receptor agonists (such as fulvestrant and tamoxifen) within 5 weeks of the FES-PET/CT scan. (Note that aromatase inhibitors and luteinizing hormone-releasing hormone agonists do not affect ER expression, or binding of FES to ER, and do not need to be discontinued or considered for inclusion or exclusion of patients).
- Patient who have had the site(s) of biopsy proven invasive lobular carcinoma surgically resected.

For Expansion Phase Added in March 2022 Amendment:

- Patients with known allergic or hypersensitivity reactions to previously administered radiopharmaceuticals. Patients with significant drug or other allergies or autoimmune diseases may be enrolled at the Investigator's discretion.
- Patients who require monitored anesthesia for PET/CT scanning.
- Patients who are too claustrophobic to undergo PET/CT scanning.
- Pregnancy or current breast feeding.
- Patient who have had the site(s) of biopsy proven invasive lobular carcinoma surgically resected. Note: *This does not apply for participants being enrolled for Exploratory Arm 1.*
- Patients undergoing treatment with estrogen receptor agonists (such as fulvestrant and tamoxifen) within 5 weeks of the FES-PET/CT scan. (Note that aromatase inhibitors and luteinizing hormone-releasing hormone agonists do not affect ER expression, or binding of FES to ER, and do not need to be discontinued or considered for inclusion or exclusion of patients). Note: *This does not apply for participants being enrolled for Exploratory Arm 1.*

4.4. Patient Registration

Participants must meet all of the eligibility requirements listed above and in Appendix A prior to registration. New patient registrations will be submitted to the clinical trials office by the study coordinator by completing a Clinical Trials Office Patient Registration Form and sending to CTORegistrations@hci.utah.edu. The study coordinator will also record study data on all patients entered into the study and complete subsequent forms.

4.5. Study Procedures, Schedule of Events, Tracer Administration: Route and Dosing

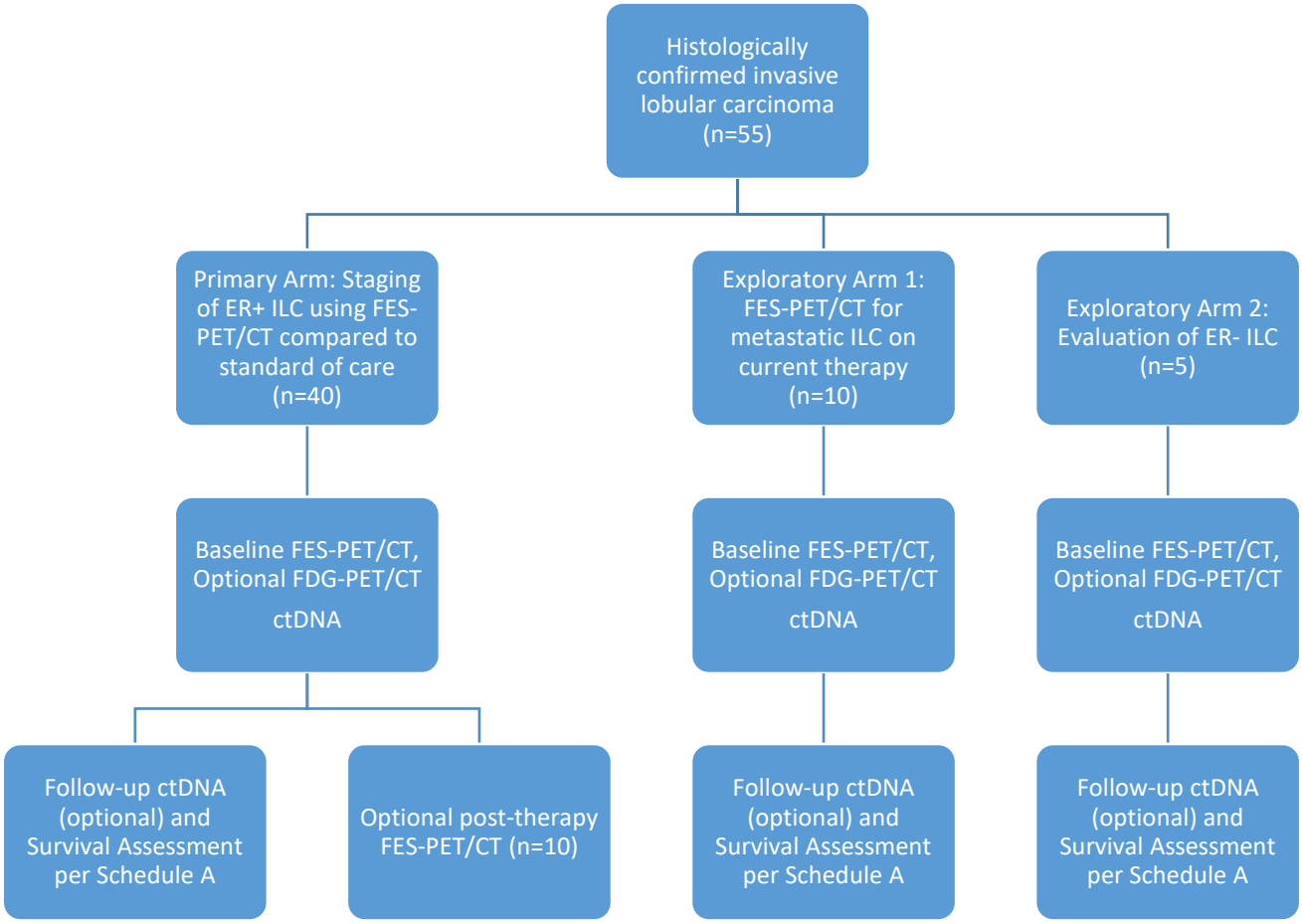
Study Schema

For Pilot Phase Completed in 2021:

Study Schema

***Note the optional [^{18}F]FDG-PET/CT session may occur before or after the [^{18}F]FES-PET/CT but may not occur on the same day**

For Expansion Phase Added in March 2022 Amendment:



4.5.1. Initial Visits Prior to PET Imaging

The patient will be evaluated by their treating oncologist or breast surgeon. The following additional patient data will be obtained: histological diagnosis (when available following biopsy or surgery), age at radiological diagnosis, gender, and other treatment modalities used. Clinical (non-research related) imaging studies, such as bone scan, contrast MRI, FDG-PET/CT, and/or standard clinical CT scans, will be collected according to standard of care. Additionally, ER status of the primary tumor, ER status of metastases (if present and if ER status is known) will be documented.

4.5.2. [^{18}F]FES–PET/CT Exams

Patient preparation prior to [^{18}F]FES-PET/CT examinations includes a recommendation but not requirement for fasting with no food or drink except for plain water for 4 or more hours prior to imaging to reduce bowel uptake due to bile excretion.

Patients who are not postmenopausal or surgically sterile must have a serum pregnancy test performed within 48 hours prior to each research PET imaging.

4.5.3. Day of Research [^{18}F]FES–PET/CT Scan (Required or Optional)

The research subject will be queried regarding recommended but optional fasting and vital signs will be obtained. Vital signs will include heart rate, blood pressure, and temperature. The patient will then have the appropriate IV access placed for radiotracer administration.

Table 5.1. PET Radiopharmaceutical Administration: Route and Dosing

Tracer	*Route*	*Manner*	*Injected Dose*	*Scan Mode*
[^{18}F]Fluoroestradiol	IV	~30s push	Target injected activity is approximately 6 mCi (allowable range of approximately 3 to 6 mCi)	Static

The [^{18}F]FES will be prepared by the PET radiochemists and radiopharmacists in the PET cyclotron facility at HCI on the day of the scanning session. The radiopharmaceutical will be administered to the patient by a physician, nuclear medicine technologist, or trained research personnel in the PET imaging suite. The [^{18}F]FES will be pushed by hand as a manually timed infusion (~30s) as indicated by Table 5.1. The uptake time for the [^{18}F]FES will be approximately 60 minutes +/- 10 minutes. The patient will be moved from the uptake room to the scanner with enough time to allow for a bathroom visit. The goal is to have the uptake time as close to 60 minutes as possible. A CT scan for PET attenuation correction and diagnostic interpretation will be acquired using x-ray CT (PET/CT scanner) from the level of the top of the head to the level of the knees. PET images will then be acquired from the top of the head to the level of the knees.

The infusion and imaging procedure will be terminated in any patient who exhibits anaphylaxis, significant hypotension (systolic blood pressure less than 80 mmHg, dyspnea, chest pain, grand mal seizure or an O_2 saturation lower than 80%.

Vital signs will be obtained again at the conclusion of the scan, as well as adverse events potentially related to the administration of [^{18}F]FES. Adverse events will also be established after approximately 24 hours but up to 72 hours. (Appendix B: Schedule of Events, Appendix C: [^{18}F]FES Infusion Flow Chart, Appendix D: [18F]FES Patient Adverse Event Questionnaire).

4.5.4. Optional [^{18}F]Fluorodeoxyglucose–PET/CT Scan

If a clinical [^{18}F]Fluorodeoxyglucose (FDG) scan has not been completed as part of standard clinical care, the patient may elect to complete an optional FDG-PET/CT, the costs of which will be covered by the study.

If performed, the optional FDG-PET/CT must be completed within 4 weeks (before or after) of the [^{18}F]FES-PET/CT study. FDG-PET/CT would be performed according to standard departmental clinical protocol. CT imaging will be performed with a non-contrast CT technique to avoid any potential for a contrast-reaction.

4.5.5. MethylPatch ctDNA analysis

After giving informed consent, subjects will undergo one phlebotomy procedure, at times specified Appenix A: Schedule of Events), to obtain at least 20 mL (two 10mL Streck BCT tubes) for methylated circulating tumor DNA (ctDNA) analysis using the MethylPatch assay. Blood specimens will be drawn by CQCI nuclear medicine research technologists and promptly delivered to HCI Biorepository and Molecular Pathology (BMP) Shared Resource for immediate isolation of plasma. Briefly, following blood collection the tube is centrifuged, and the plasma supernatant is collected by carefully excluding the buffy coat containing leukocytes. The plasma is centrifuged a second time to remove trace cells and macromolecules, and 4-5 mL of supernatant plasma is aliquoted into cryovials for long-term frozen storage. Separate aliquots of buffy coat and plasma specimens will be encoded with a de-identified study identifier (Shadow ID & CC#) and stored in the BMP freezers for later disbursement. BMP will act as the honest broker to maintain the separation of PHI from the de-identified patient specimens. This procedure will be repeated at the prescribed study intervals (described in Appendix A: Schedule of Events).

The Varley Lab will submit disbursement requests to BMP for the de-identified plasma specimens at regular intervals for MethylPatch analysis. The first step of this analysis involves cfDNA extraction using the MagMAX Cell-Free DNA Isolation Kit. The quantity and quality of each cfDNA sample is assessed to ensure it meets critical metrics (> 40 ng, >30% 150-250bp in size). If the cfDNA meets these criteria, then series of molecular biology reactions are performed to enrich for 54 biomarker regions of DNA and to convert unmethylated cytosines in the DNA sequence to uracil to reveal the methylation status of the DNA (enzymatic conversion). The DNA is then amplified by polymerase chain reaction and sequenced on an illumina Nova-seq instrument. Bioinformatics analysis of the sequencing results is used to determine if the results meet critical quality metrics: 80% of the biomarkers have at least 1,000 molecules with >=5x read depth, and enzymatic conversion efficiency >98%. Bioinformatics analysis is then performed on each sample that meets these criteria to quantify methylated ctDNA. The quantity of methylated ctDNA is reported for each de-identified sample as the percentage of molecules from each biomarker that were methylated, and the mean percentage of methylated molecules. The MethylPatch assay returns a positive result when at least 3 methylated breast cancer biomarker regions are identified and the mean percent methylation across biomarkers is greater than 0.15%. Statistical analysis will be used to evaluate the correlation between the quantity of methylated ctDNA detected in each

specimen and patient stage, tumor FES-PET/CT uptake, progression free survival, and overall survival.

To support the exploratory objectives and correlative studies, blood samples will be collected at the time points indicated on the Schedule of Events. After completion of the MethylPatch PCR ctDNA assay, any remaining blood will be stored for future unspecified cancer research. With the participant's approval, and as approved by the Institutional Review Board (IRB), de-identified biological samples will be stored at Huntsman Cancer Institute's Biorepository.

At the time of consent, subjects will be consented as part of enrollment to authorize the biobanking of any residual blood sample for use in future undisclosed cancer research that may include other types of ctDNA analysis. During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, the withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

Blood Samples:
Up to 40 mLs of blood will be collected at the time-points indicated on the Schedule of Events. When samples are due to be collected on the same day as treatment administration, samples should be collected prior to study therapy.

4.6. Data Collection

4.6.1. Safety Data Recorded During [^{18}F]Fluoroestradiol-PET/CT Scans

Data collected on the visit for [^{18}F]Fluoroestradiol-PET/CT scanning will be recorded on the [^{18}F]Fluoroestradiol Protocol flow chart and will include: vital signs (including heart rate, blood pressure and temperature) recorded just prior to the [^{18}F]Fluoroestradiol infusion and then after the completion of the [^{18}F]Fluoroestradiol-PET/CT imaging study. In addition to monitoring the above-mentioned safety parameters, an emergency code cart is located in the PET suite and can be utilized by the PET imaging team, as well as accessed by the code team at the institution should an emergent situation arise.

Additional information to be collected will include:

- Questioning of patient regarding adverse events immediately after [^{18}F]FES administration.
- Patients may be discharged after the completion of the [^{18}F]FES-PET/CT scan if stable and if no adverse events are noted on questioning. Patients will be evaluated for adverse events after the completion of the [^{18}F]Fluoroestradiol-PET/CT scan and at approximately 24 hours

4.6.2. Tissue Collection and Analysis

All patients enrolled on this study will have undergone a procedure (either biopsy or tumor resection) in order to obtain a histological diagnosis of their tumors as part of standard care. The surgical pathology report will be reviewed and the pathologic stage entered into the research database. Other pathologic assessment results including immunohistochemistry of receptor status will also be entered into the research database.

5. METHODS FOR EVALUATION OF IMAGING STUDIES

Each PET imaging study will be evaluated using qualitative visual assessment and semi-quantitative analysis techniques that have previously been used by us and other groups, and represent the standard and accepted means of evaluating static PET images. These assessments are described below.

5.1. [^{18}F]FES-PET/CT Imaging

5.1.1. [18F]FES Qualitative Visual Assessment

The uptake of [^{18}F]Fluoroestradiol is mediated by binding of the [^{18}F]Fluoroestradiol to the estrogen receptors which are found in approximately 70-75% of invasive breast cancers overall and greater than 90% of invasive lobular carcinomas [14, 34, 77]. The ability of [^{18}F] Fluoroestradiol-PET to detect malignant lesions in humans has been assessed in studies by researchers at Washington University in St. Louis, University of Pennsylvania, and others [34, 38, 42-47, 76, 78-87].

Visual assessment will be used to qualitatively assess for abnormal focal tracer uptake throughout the imaged body. Images will be reviewed in an unblinded manner to assess whether additional lesions are found when comparing with prior imaging.

5.1.2. [^{18}F]FES-PET Semi-Quantitative Assessment

[^{18}F]FES uptake will be quantified by calculating body-weight-corrected Standardized Uptake Values (SUVmax) as follows:

$$SUV_{max} = \frac{A_{max}}{injected\ activity\ (mCi)/_{BW}}$$

where A_{max} = radioactive concentration (mCi/mL)
BW = body weight BW (g)

[88]

The maximum SUV (SUV_{max}) and mean SUV (SUV_{mean}) on the lesions identified on the qualitative visual analysis, with cutoff for abnormal FES accumulation of the body-weight-corrected SUV_{max} greater or equal to 1.5, a cutoff which has been used in prior studies. A region-of-interest (ROI) will be drawn over the lesions (on all image slices

with abnormal uptake). The slice values will then be averaged to obtain an SUV_{mean}. Each lesional SUV_{max} and SUV_{mean} value will then be averaged to obtain the average SUV_{max} and average SUV_{mean} values on a per-person basis and also used for further analysis.

5.2 [^{18}F]Fluorodeoxyglucose PET Imaging

5.2.1. [^{18}F]Fluorodeoxyglucose Qualitative Visual Assessment

Visual assessment will be used to qualitatively assess for abnormal focal tracer uptake above background for the same type of tissue throughout the imaged body. Images will be reviewed in an unblinded manner to assess whether additional lesions are found when comparing with prior imaging.

5.2.2. [^{18}F]Fluorodeoxyglucose Semi-Quantitative Assessment

Semi-quantitative assessment of [^{18}F]Fluorodeoxyglucose-PET uptake will be performed by calculating SUV_{max} and SUV_{mean} for each lesion in the same manner as described for FES-PET in section 5.1.2.

6. DATA ANALYSIS AND STATISTICS

6.1. Validation of [^{18}F]Fluoroestradiol-PET/CT Imaging to Assess the Uptake in Invasive Lobular Carcinoma

For Pilot Phase Completed in 2021

This study will provide preliminary exploratory data in the value [^{18}F]FES to assess the avidity of invasive lobular carcinoma for both estrogen receptor positive and estrogen receptor negative cases.

For Expansion Phase, as of March 2022 Amendment

The study will provide exploratory data regarding use of [^{18}F]FES-PET/CT for staging of patients with ILC, and for FES-PET/CT imaging in patients on endocrine therapies. The study will also provide exploratory data regarding use of MethylPatch PCR ctDNA assay for ILC staging and surveillance.

6.2 Data Analysis

For Pilot Phase Completed in 2021

Qualitative and Visual Assessment. The images for the [^{18}F]Fluoroestradiol-PET/CT will be visually assessed for any artifacts. Images will then be assessed for presence of FES avidity in the biopsy proven invasive lobular carcinoma and in any other sites throughout the body. Data will then be assessed and tabulated on a lesion by lesion basis as to the presence of abnormal FES-PET uptake.

Semi- and Fully-Quantitative Assessments.

Primary endpoint:

1. The primary endpoint is the positive uptake rate of invasive lobular carcinoma on FES-PET/CT (yes/no whether the tumor shows positive uptake on FES PET). The null hypothesis is that 60% will show positive uptake. The null hypothesis will be tested using a one-sided test of binomial proportions at Type I error (alpha) = 0.10. A 95% exact binomial confidence interval for positive uptake will also be reported.

Secondary endpoints:

1. Rate of estrogen receptor positive (ER+) ILC that does not demonstrate positive FES uptake, defined as focal uptake above background with SUV max of 1.5 or greater. The proportion of ER+ ILC that does not demonstrate positive FES uptake will be reported along with a 95% exact binomial confidence interval.

2. Rate of estrogen receptor negative (ER-) ILC that does demonstrate positive FES uptake, defined as focal uptake above background with SUV max of 1.5 or greater. The proportion of ER- ILC that demonstrate positive FES uptake will be reported along with a 95% exact binomial confidence interval.

3. Rate of same-patient (inter-tumoral) heterogeneous FES uptake defined as presence of FES uptake in some but not all biopsy proven or suspected metastatic lesions. The proportion of patients with heterogeneous FES update will be reported along with a 95% exact binomial confidence interval.

4. For cases with both FDG- and FES-PET/CT imaging, evaluate the rate of discordant uptake (FES positive/FDG negative or FES negative/FDG positive). Discordant uptake will be tabulated and evaluated using McNemar's test for correlated proportions.

5. For cases with both [^{18}F]Fluoroestradiol- PET/CT and [^{18}F]FDG-PET/CT studies, Spearman correlation of lesion uptake on [^{18}F]Fluoroestradiol- PET/CT and [^{18}F]FDG-PET/CT will be calculated. The number of lesions identified by [^{18}F]Fluoroestradiol-PET/CT alone, and [^{18}F]FDG-PET/CT alone, and both [^{18}F]Fluoroestradiol- PET/CT and [^{18}F]FDG-PET/CT will be tabulated.

For Expansion Phase, as of March 2022 Amendment

Patients in the Primary Arm (see Study Schema in Section 4.5) will contribute to the analysis of Primary and Secondary Endpoints.

Primary Endpoint: Overall percentage of change of stage (Yes/No) following research FES-PET/CT imaging compared to stage based on standard of care imaging.

The primary endpoint will analyzed using a one-sided one-sample test of binomial proportions at one-sided alpha = 0.05. The null hypothesis (H0) is that the proportion of patients with a change in staging is 5% or less. The alternative hypothesis (H1) is that the proportion of patients with a change in staging is 20% or more.

Secondary Endpoint 1: Correlation between methylated DNA and stage at presentation.

Spearman correlation will be used to assess the correlation between ctDNA and stage.

Secondary Endpoint 2: The relationship between the quantity of methylated ctDNA and overall survival.

A proportional hazards model will be used to assess the relationship between methylated ctDNA and overall survival. To determine the relationship at various time points, the analysis will be performed with censoring at 6, 12, 18, 24, 36, 48 and 60 months.

Secondary Endpoint 3: Relationship between the presence of heterogeneous FES-PET/CT uptake at baseline (binary variable/yes/no) and overall survival.

Kaplan-Meier methods and a log rank test will be used to assess the relationship between FES-PET/CT and overall survival.

Exploratory Endpoint 1: The rate of complete ER blockade (yes/no) at 2-4 weeks post-therapy in ER+ ILC patients. This endpoint will be restricted to patients in the primary arm with ER+ ILC that complete an optional post-therapy FES-PET/CT.

The number and proportion of sites with persistent abnormal FES uptake will be reported along with a 95% exact binomial confidence interval. Resampling methods may be used to report a confidence interval adjusted for possible correlation between multiple lesions in the same patient.

Exploratory Endpoint 2: The rate of persistent abnormal FES uptake (yes/no) in metastatic patients on hormonal therapy with known ER+ ILC. This endpoint will be restricted to metastatic patients with ER+ ILC in Exploratory Arm 1 that complete an optional post-therapy FES-PET/CT.

The number and proportion of sites with persistent abnormal FES uptake will be reported along with a 95% exact binomial confidence interval. Resampling methods

may be used to report a confidence interval adjusted for possible correlation between multiple lesions in the same patient.

Exploratory Endpoint 3: The negative predictive value for negative (no abnormal uptake) FES-PET/CT imaging with estrogen receptor (ER) negative ILC as determined by immunohistochemistry from core needle biopsy. This endpoint will be restricted to patients in Exploratory Arm 2 with estrogen receptor (ER) negative ILC.

The negative predictive value will be reported together with a 95% exact binomial confidence interval. Resampling methods may be used to report a confidence interval adjusted for possible correlation between multiple lesions in the same patient.

Exploratory Endpoint 4: The relationship between heterogeneous FES-PET/CT uptake at baseline (yes/no) and development of metastatic disease during follow-up. This endpoint will be restricted to patients in the Primary Arm and Exploratory Arm 2 who have no distant metastatic disease at baseline.

Kaplan-Meier methods will be used to estimate the proportion of non-metastatic patients that develop metastatic disease at 6, 12, 18, 24, 36, 48 and 60 months. This will be done separately in patients with and without heterogeneous FES-PE/CT uptake. A log-rank test will be used to compare the two groups.

Exploratory Endpoint 5: The relationship between heterogeneous FES-PET/CT uptake at baseline (yes/no) and development of new metastatic disease during follow-up. This endpoint will be restricted to patients in all study arms who have metastatic disease at baseline.

Kaplan-Meier methods will be used to estimate the proportion of metastatic patients that develop new metastatic disease at 6, 12, 18, 24, 36, 48 and 60 months. This will be done separately in patients with and without heterogeneous FES-PE/CT uptake. A log-rank test will be used to compare the two groups.

Exploratory Endpoint 6: The relationship between the quantity of methylated ctDNA at baseline and development of recurrent disease (yes/no) for patients with no distant metastatic disease at baseline. This endpoint will be restricted to subjects in the Primary Arm and Exploratory Arm 2 with no distant metastatic disease.

Logistic regression will be used to assess the relationship between methylated ctDNA and recurrent disease.

Exploratory Endpoint 7: The relationship between the quantity of methylated ctDNA at baseline and development of new metastatic disease (yes/no) for patients with metastatic disease at baseline. This endpoint will be restricted to subjects in all arms with metastatic disease at baseline.

Logistic regression will be used to assess the relationship between methylated ctDNA and recurrent disease.

6.3. Justification of Sample Size

For Pilot Phase Completed in 2021

The initial primary endpoint from the completed pilot study was to establish the positive uptake rate of invasive lobular carcinoma on FES-PET/CT (yes/no whether the tumor shows positive uptake on FES-PET). The null hypothesis is that 60% will show positive uptake, similar to FDG-PET/CT. A higher proportion of tumors are expected to have positive uptake by FES-PET/CT. The null hypothesis will be tested using a one-sided test of binomial proportions at Type I error (alpha) = 0.10. With data from 24 tumors there will 81% power provided the true proportion that show positive uptake is 80%. The null hypothesis will be rejected if at least 18/24 tumors show positive FES-PET/CT uptake.

For Expansion Phase, as of March 2022 Amendment

The revised primary endpoint is the change in tumor stage when staged with FES-PET/CT compared to current standard of care imaging. For the primary statistical analysis, change in staging with FES-PET/CT will be coded as a binary variable (changed, unchanged). The null hypothesis (H0) is that the proportion of patients with a change in staging is 5% or less. The alternative hypothesis (H1) is that the proportion of patients with a change in staging is 20% or more. With these hypotheses, 38 evaluable patients will provided 90% power using a one sample exact binomial test at one-sided alpha = 0.05. H0 will be rejected if at least 5 patients have a change in staging. For the primary endpoint 40 patients will be recruited to allow 2 redundant patients to ensure that at least 38 evaluable patients will be available for statistical review. An additional 17 patients may be enrolled to achieve the exploratory aims of this study.

7. REGULATORY AND REPORTING REQUIREMENTS

The AE reporting time frame for this study's investigational radiopharmaceutical is 24 hours post injection. Per the approval of IND #151981 for [^{18}F]FES the following reporting of unexpected fatal or life threatening events, serious adverse events, and serious and unexpected adverse events will occur: (1) Reporting any unexpected fatal or life threatening adverse experience associated with the use of [^{18}F]FES by telephone or fax no later than 7 calendar days after initial receipt of the information. (2) Reporting any adverse experience associated with the use of [^{18}F]Fluoroestradiol, that is both serious (SAE) and unexpected in writing no later than 15 calendar days after initial receipt of the information. (3) Submitting annual reports. The reportable events will also

be submitted to the Institutional Review Board (IRB) using the University of Utah ERICA online system:
https://erica.research.utah.edu/erica/Rooms/DisplayPages/LayoutInitial?Container=com.webridge.entity.Entity%5BOID%5B5FD2DA60262617429607E459C0E09D92%5D%5D

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 with subsequent modifications will be utilized for adverse event reporting (http://ctep.cancer.gov/reporting/index.html).

All appropriate treatment areas will have access to a copy of the CTCAE version 5.0 with modifications. A list of adverse events that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in Section 3 (Toxicity of [^{18}F]FES in Humans)

7.1. Human Subject Protections

The study will be conducted in accordance with the appropriate FDA, IRB, ICH GCP, and other federal and local regulatory requirements, as applicable. Informed consent will be obtained from all research participants prior to performing any study procedures using the most recent IRB-approved version. All patients must be at least 18 years of age to participate.

7.2 Institutional Review

Before study initiation, the investigator must have written and dated approval/favorable opinion from the IRB/IEC for the protocol, consent form, subject recruitment materials (e.g., advertisements), and any other applicable patient-facing documents. The investigator should also provide the IRB/IEC with a copy of the Investigator Brochure or product labeling information.

The investigator or designee should provide the IRB/IEC with reports, updates and other information (eg, expedited safety reports, amendments, and administrative letters) according to regulatory requirements or institution procedures.

7.3 Data and Safety Monitoring Plan

A Data and Safety Monitoring Committee (DSMC) is established at Huntsman Cancer Institute (HCI) and approved by the NCI to assure the well-being of patients enrolled on Investigator Initiated Trials that do not have an outside monitoring review. Roles and responsibilities of the DSMC are set forth in the NCI approved plan. The activities of this committee include review of adverse events including SAEs, important medical events, significant revisions or amendments to the protocol, and approval of cohort/dose escalations. If the DSMC and/or the PI have concerns about unexpected safety issues, the study will be stopped and will not be resumed until the issues are resolved. The DSMC also reviews and approves audit reports generated by the Research Compliance Office.

This study is classified as low risk per the NCI-approved DSM plan. Each low-risk study may be assigned a physician member of the DSMC as medical monitor, or in rare cases, an external medical monitor. The medical monitor will be notified of all serious adverse events (SAEs). SAEs occurring in patients treated at HCI or its affiliates will also be reviewed by the full DSMC monthly.
Low-risk trials will be monitored by RCO personnel after the first patient is enrolled. Following the initial monitoring visit, the RCO will conduct audits for low-risk studies annually thereafter. Audits of low-risk studies may be conducted more frequently as requested by the DSMC, PRMC, IRB, RCO management, or the PI. Low-risk trials will be formally reviewed by the DSMC after the first patient is enrolled and then annually thereafter.

7.3.1 Data Reporting

This study will be monitored by the Principal Investigator using the ERICA system. In addition, the study will be monitored by the HCI Data and Safety Monitoring Committee. Cumulative data will be submitted electronically to ERICA, the HCI Data and Safety Monitoring Committee, and the IRB as required.

7.4 Adverse Events / Serious Adverse Events

This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) version 5.0 for AE and SAE reporting. An electronic copy of the CTCAE version 5.0 can be downloaded from:
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50

7.4.1 Adverse Events (AE)

An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after receiving the radioactive tracer(s) and 24 hours afterword even if the event is not considered to be related to the tracer. For the purposes of this study, the terms toxicity and adverse event are used interchangeably. Medical conditions/diseases present before starting the study are only considered adverse events if they worsen after being injected with the radiopharmaceutical(s). Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

The collection of any adverse events will begin when a patient receives their first dose of [^{18}F]FES and will end 24 hours after receiving that dose. Adverse event monitoring and reporting following administration of [^{18}F]FDG is not necessary for this protocol as this is a clinically available, non-investigational imaging agent.

Information about all adverse events, whether volunteered by the subject, discovered by the investigator questioning, or detected through physical examination, laboratory test

or other means, will be collected and recorded and followed as appropriate. Those adverse events that are not associated with [^{18}F]FES that do not require expedited reporting will be reported in the routine manner to the IRB.

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit or phone contact during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory tests, or other assessments. As far as possible, each adverse event should be evaluated to determine:

1. The severity grade based on CTCAE v.5 (Grade 1-5).
2. Its relationship to the study radioactive tracer(s) (definite, probably, possible, unlikely, not related)
3. Its duration (start and end dates or if continuing at final exam).
4. Action taken (no action taken; study tracer dosage adjusted/temporarily interrupted; study tracer permanently discontinued due to this adverse event; concomitant medication taken; non-drug therapy given; hospitalization/prolonged hospitalization).
5. Whether it constitutes an SAE.

All adverse events will be treated appropriately. Once an adverse event is detected, it should be followed until its resolution, and assessment will include any changes in severity, the suspected relationship to the study tracer, the interventions required to treat it, and the outcome.

Information about common side effects already known about the tracer is described in the Pharmacology and Safety of [^{18}F]FES and Radiation Dosimetry of [^{18}F]FES (Section 3). This information will be included in the patient informed consent and will be discussed with the patient during the study as needed.

All adverse events will be immediately recorded in the patient research chart.

7.4.2 Serious Adverse Event (SAE)

Information about all serious adverse events will be collected and recorded during the 24 hour reporting period. A serious adverse event is an undesirable sign, symptom, or medical condition which:

- Is fatal or life-threatening.
- Results in persistent or significant disability/incapacity.
- Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above.
- Causes congenital anomaly or birth defect.
- Requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:

- Routine treatment or monitoring of the studies indication, not associated with any deterioration in condition (procedures such as central line placements, paracentesis, and pain control).
- Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug.
- Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission.
- Social reasons and respite care in the absence of any deterioration in the patient's general condition.

Any death from any cause while a patient is receiving tracer on this protocol or up to 30 days after the administration of a radioactive tracer(s), or any death which occurs more than 30 days after administration of a tracer(s) has ended but which is felt to be related to the tracer, must be reported.

Note: All deaths on study will be reported 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 IRB within 24 hours of notification of the event, indicating that a full report will follow. Any unexpected fatal or life threatening adverse experience associated with the use of [^{18}F]Fluoroestradiol will be reported to the FDA by telephone or fax no later than 7 calendar days after initial receipt of the information. All reportable adverse events will be submitted to the FDA & IRB within the required timeframe by as mandated by the FDA and IRB.

Toxicities which fall within the definitions listed above must be reported as an SAE regardless if they are felt to be related to the radioactive tracer(s) or not. Toxicities unrelated to the radioactive tracer(s) that do NOT fall within the definitions above, must simply be documented as AEs in the patient research chart.

7.5 SAE Reporting Requirements

SAEs must be reported to the DSMC, the FDA, and the IRB, according to the requirements described below:

A MedWatch 3500 A form must be completed and submitted to compliance@hci.utah.edu as soon as possible, but no later than 10 days of first knowledge or notification of event (5 days for fatal or life threatening event).

*MedWatch 3500A form can be found at:
http://www.fda.gov/dowloads/Safety/MedWatch/HowToReport/DowloadForms/ucm082728.pdf

DSMC Notifications:

- An HCI Research Compliance Officer (RCO) will process and submit the MedWatch form to the proper DSMC member as necessary for each individual study.
- The RCO will summarize and present all reported SAEs according to the Data and Safety Monitoring Plan at the quarterly DSMC meeting.

FDA Notifications:

- Adverse events occurring during the course of a clinical study that meet the following criteria will be promptly reported to the FDA:
    - Serious
    - Unexpected
    - Definitely, Probably, or Possibly Related to the investigational drug
    - Fatal or life-threatening events that meet the criteria above will be reported within 7 calendar days after first knowledge of the event by the investigator; followed by as complete a report as possible within 8 additional calendar days.
    - All other events that meet the criteria above will be reported within 15 calendar days after first knowledge of the event by the investigator.
    - The RCO will review the MedWatch report for completeness, accuracy and applicability to the regulatory reporting requirements.
    - The RCO will ensure the complete, accurate and timely reporting of the event to the FDA.
    - The Regulatory Coordinator will submit the report as an amendment to the IND application.
    - All other adverse events and safety information not requiring expedited reporting that occur or are collected during the course of the study will be summarized and reported to the FDA through the IND Annual Report.

IRB Notification:

- Events meeting the University of Utah IRB reporting requirements (http://www.research.utah.edu/irb/) will be submitted through the IRB's electronic reporting system within 10 working days.

7.6 Protocol Amendments

Any amendments or administrative changes in the research protocol during the period, for which the IRB approval has already been given, will not be initiated without submission of an amendment for IRB review and approval.

These requirements for approval will in no way prevent any immediate action from being taken by the investigator in the interests of preserving the safety of all patients included in the trial.

Any amendments to the protocol that significantly affect the safety of subjects, scope of the investigation, or the scientific quality of study are required to submit the amendment for FDA review.

7.7 Protocol Deviations

A protocol deviation (or violation) is any departure from the defined procedures and treatment plans as outlined in the protocol version submitted and previously approved by the IRB. Protocol deviations have the potential to place participants at risk and can also undermine the scientific integrity of the study thus jeopardizing the justification for the research. Protocol deviations are unplanned and unintentional events.

Because some protocol deviations pose no conceivable threat to participant safety or scientific integrity, reporting is left to the discretion of the PI within the context of the guidelines below. The IRB requires the **prompt reporting** of protocol deviations which are:

- Exceptions to eligibility criteria.
- Intended to eliminate apparent immediate hazard to a research participant or
- Harmful (caused harm to participants or others, or place them at increased risk of harm – including physical, psychological, economic, or social harm), or
- Possible serious or continued noncompliance.

7.8 FDA Annual Reporting

An annual progress report will be submitted to the FDA within 60 days of the anniversary of the date that the IND went into effect. (21 CFR 312.22).

7.9 Clinical Trials Data Bank

The study will be registered on http://clinicaltrials.gov and the NCI CTRG (Clinical Trials Reporting Program) by the Clinical Trials Office.

7.10 Record Keeping

Per 21 CFR 312.57, Investigator records shall be maintained for a period of 2 years following the date a marketing application is approved; or, if no application is filed or the application is not approved, until 2 years after the investigation is discontinued and the FDA is notified.

8. PET RADIOPHARMACEUTICAL PRODUCTION

8.1. [^{18}F]Fluoroestradiol

8.1.1. Study Agent

[^{18}F]Fluoroestradiol is a fluorine-18 labelled PET radiopharmaceutical supplied as a ready-to-inject solution in either vials or syringes. The maximum dose volume is 5 ml. The drug substance is [^{18}F]Fluoroestradiol. The formulation of [^{18}F]FES drug product contains saline, 0.9% (v/v) sodium phosphates for injection and no more than 10% (v/v) ethanol. The pH of the drug product is 6-8. The radiochemical purity (RCP) is greater than or equal to 95% throughout the shelf-life (up to 6 hours). [^{18}F]Fluoroestradiol injection is manufactured by automated radiosynthesis followed by formulation with buffer and aseptic dispensing in a remotely controlled system. Fluorine-18 decays by

positron emission (β+ decay, 96.7%) and orbital electron capture (3.3%) with a half-life of approximately 110 minutes (mins). The positron undergoes annihilation with an electron to produce two gamma photons each of energy 511 keV (193.4% emission).

[^{18}F]Fluoroestradiol injection is a sterile, aqueous solution of [^{18}F]FES and excipients for intravenous administration. The product dose is supplied with a radioactive content of 111-222 MBq/ml (3-6 mCi/ml) at the requested calibration time and time in an aseptically prepared syringe or a glass vial sealed with a synthetic rubber closure and aluminum overseal and then withdrawn into syringes at the clinical site. Each vial or syringe is transported in a lead or tungsten shield. The quality control (QC) analysis of a sample of the drug product as well as product release for human use is completed by a qualified quality assurance team before transportation of the drug product to the study site. The investigator (or nominated deputy) will receive release/reject information for the drug product. Only product for which confirmation of release has been received shall be used. Where the product is transported as a single patient dose, confirmation of the dose will be measured in a dose calibrator before administration. The calculation is based on the radioactive content, the half-life of fluorine-18 (109.8 mins), the reference date and time, the prescribed dose and the time of injection. Each patient dose will contain between 111-222 MBq (3.0-6.0 mCi) at the time of administration. The doses will contain no more than 5 µg [^{18}F]FES and 5 ug related substances per injection. The maximum administered dose volume is 5 ml.

8.1.2. Reported Adverse Events and Potential Risks

No serious adverse reactions have been reported from any published studies in part 3.1.2 of this document. The risks to subjects mainly relate to the intravenous injection and intravenous blood sampling procedures, and the radiation emitted by [^{18}F]Fluoroestradiol. Intravenous injection and the use of an intravenous cannula are known to carry a small risk of infection and hematoma. The exposure to radiation will not exceed that which is considered acceptable in accordance with appropriate guidelines.

8.1.3. Production of the Radiopharmaceutical

The [^{18}F]FES used in this study will be prepared locally by the PET Radiochemistry Group at the University of Utah. The precursors for the radiosynthesis include F-18 prepared at the Huntsman Cancer Institute cyclotron from proton irradiation of [O-18] water and an organic precursor supplied along with other reagents are used on the TRACERlab FX-N synthesis module. [^{18}F]Fluoroestradiol is manufactured by automated radiosynthesis on the TRACERlab FX-N followed by formulation with buffer and aseptic dispensing in a remotely controlled system. The formulation of [^{18}F]FES drug product contains sodium phosphates buffer in saline. The pH of the drug product is 6-8. The radiochemical purity (RCP) is greater than or equal to 95% throughout the shelf-life (up to 6 hours). The radiopharmaceutical product is a clear and colorless liquid that is stored at room temperature in a sterile serum vial. The [^{18}F]FES currently has an expiration time of 6 hours from the end of synthesis (EOS).

8.1.4. Agent Accountability

[^{18}F]Fluoroestradiol is a radiopharmaceutical produced in the cyclotron facility at the Huntsman Cancer Institute. The agent is investigational and approved by the FDA under IND#151981 (Hoffman).

The shelf-life of [^{18}F]FES is up to 6 hours from the end of synthesis and the product must not be used beyond this limit. [^{18}F]Fluoroestradiol should be stored at room temperature (~15-25°C) in a shielded container. All non-radioactive containers (shielding, transport cans) must be returned to the manufacturing site. Containers that are radioactive or that contained radioactive products must be destroyed at either the study site or another designated facility, after the study and after overall drug accountability has been completed by the sponsor or its representative. Waste must be disposed of according to national regulations for radioactive material. Precautions for the safe handling of radioactive materials should be observed.

Each radiosynthesis is done by University of Utah cyclotron and radiochemistry staff and the product [^{18}F]Fluoroestradiol in a dose calibrated syringe will be released after passing all required quality control assays to the physician who will be responsible for administering the appropriate amount (John M. Hoffman, MD or his designee). The quality control tests that must be passed prior to release of the product [^{18}F]FES for injection include the radioactive purity, the radiochemical purity, sterilizing filter integrity impurities tests, pyrogens and appearance. The [^{18}F]FES dose is drawn into a syringe, assayed for mCi at the time of injection, and administered to the research subject.

9. REFERENCES

1. Hogan, M.P., et al., *Comparison of 18F-FDG PET/CT for Systemic Staging of Newly Diagnosed Invasive Lobular Carcinoma Versus Invasive Ductal Carcinoma.* J Nucl Med, 2015. **56**(11): p. 1674-80.
2. Fujii, T., et al., *Clinical Significance of 18F-FDG-PET in Invasive Lobular Carcinoma.* Anticancer Res, 2016. **36**(10): p. 5481-5485.
3. Avril, N., et al., *Breast imaging with positron emission tomography and fluorine-18 fluorodeoxyglucose: use and limitations.* J Clin Oncol, 2000. **18**(20): p. 3495-502.
4. Berg, W.A., et al., *Diagnostic accuracy of mammography, clinical examination, US, and MR imaging in preoperative assessment of breast cancer.* Radiology, 2004. **233**(3): p. 830-49.
5. Lopez, J.K. and L.W. Bassett, *Invasive lobular carcinoma of the breast: spectrum of mammographic, US, and MR imaging findings.* Radiographics, 2009. **29**(1): p. 165-76.
6. Jung, N.Y., et al., *Associations between the standardized uptake value of (18)F-FDG PET/CT and the prognostic factors of invasive lobular carcinoma: in comparison with invasive ductal carcinoma.* World J Surg Oncol, 2015. **13**: p. 113.
7. Bevers, T.B., et al., *Breast Cancer Screening and Diagnosis, Version 3.2018, NCCN Clinical Practice Guidelines in Oncology.* J Natl Compr Canc Netw, 2018. **16**(11): p. 1362-1389.
8. Riedl, C.C., et al., *Retrospective analysis of 18F-FDG PET/CT for staging asymptomatic breast cancer patients younger than 40 years.* J Nucl Med, 2014. **55**(10): p. 1578-83.
9. Fuster, D., et al., *Preoperative Staging of Large Primary Breast Cancer With [18F]Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography Compared With Conventional Imaging Procedures.* Journal of Clinical Oncology, 2008. **26**(29): p. 4746-4751.
10. van der Hoeven, J.J., et al., *18F-2-fluoro-2-deoxy-d-glucose positron emission tomography in staging of locally advanced breast cancer.* J Clin Oncol, 2004. **22**(7): p. 1253-9.
11. Groheux, D., et al., *Prognostic impact of (18)FDG-PET-CT findings in clinical stage III and IIB breast cancer.* J Natl Cancer Inst, 2012. **104**(24): p. 1879-87.
12. Dashevsky, B.Z., et al., *Appearance of untreated bone metastases from breast cancer on FDG PET/CT: importance of histologic subtype.* Eur J Nucl Med Mol Imaging, 2015. **42**(11): p. 1666-1673.
13. Heudel, P., et al., *Value of PET-FDG in primary breast cancer based on histopathological and immunohistochemical prognostic factors.* Int J Clin Oncol, 2010. **15**(6): p. 588-93.
14. Xiao, Y., et al., *Mixed invasive ductal and lobular carcinoma has distinct clinical features and predicts worse prognosis when stratified by estrogen receptor status.* Sci Rep, 2017. **7**(1): p. 10380.
15. Hilleren, D.J., et al., *Invasive lobular carcinoma: mammographic findings in a 10-year experience.* Radiology, 1991. **178**(1): p. 149-54.
16. Avril, N., et al., *Glucose metabolism of breast cancer assessed by 18F-FDG PET: histologic and immunohistochemical tissue analysis.* J Nucl Med, 2001. **42**(1): p. 9-16.

17. Buck, A., et al., *FDG uptake in breast cancer: correlation with biological and clinical prognostic parameters.* Eur J Nucl Med Mol Imaging, 2002. **29**(10): p. 1317-23.
18. Bos, R., et al., *Biologic correlates of (18)fluorodeoxyglucose uptake in human breast cancer measured by positron emission tomography.* J Clin Oncol, 2002. **20**(2): p. 379-87.
19. Gil-Rendo, A., et al., *Association between [18F]fluorodeoxyglucose uptake and prognostic parameters in breast cancer.* Br J Surg, 2009. **96**(2): p. 166-70.
20. Ueda, S., et al., *Clinicopathological and prognostic relevance of uptake level using 18F-fluorodeoxyglucose positron emission tomography/computed tomography fusion imaging (18F-FDG PET/CT) in primary breast cancer.* Jpn J Clin Oncol, 2008. **38**(4): p. 250-8.
21. Kumar, M., et al., *(18)F-Fluoroestradiol Positron Emission Tomography Imaging of Activating Estrogen Receptor Alpha Mutations in Breast Cancer.* J Nucl Med, 2019.
22. Jones, E.F., et al., *Initial experience of dedicated breast PET imaging of ER+ breast cancers using [F-18]fluoroestradiol.* NPJ Breast Cancer, 2019. **5**: p. 12.
23. Chae, S.Y., et al., *Diagnostic accuracy and safety of 16α-[18F]fluoro-17β-oestradiol PET-CT for the assessment of oestrogen receptor status in recurrent or metastatic lesions in patients with breast cancer: a prospective cohort study.* The Lancet Oncology, 2019. **20**(4): p. 546-555.
24. Yamane, T., et al., *18F-Fluoroestradiol PET/CT Correctly Diagnosed 18F-FDG-Avid Inflammatory Lymph Nodes in a Patient With Estrogen Receptor-Positive Breast Cancer.* Clin Nucl Med, 2018. **43**(5): p. 379-380.
25. Nienhuis, H.H., et al., *(18)F-Fluoroestradiol Tumor Uptake Is Heterogeneous and Influenced by Site of Metastasis in Breast Cancer Patients.* J Nucl Med, 2018. **59**(8): p. 1212-1218.
26. Liu, S., et al., *The feasibility of (18)F-FES and (18)F-FDG microPET/CT for early monitoring the effect of fulvestrant on sensitizing docetaxel by downregulating ERalpha in ERalpha+ breast cancer.* Ann Nucl Med, 2018. **32**(4): p. 272-280.
27. Linden, H.M., L.M. Peterson, and A.M. Fowler, *Clinical Potential of Estrogen and Progesterone Receptor Imaging.* PET Clinics, 2018. **13**(3): p. 415-422.
28. Kurland, B.F., et al., *FDG PET and FES PET Predict PFS on Endocrine Therapy-Response.* Clin Cancer Res, 2018. **24**(1): p. 249-250.
29. Koleva-Kolarova, R.G., et al., *Molecular imaging with positron emission tomography and computed tomography (PET/CT) for selecting first-line targeted treatment in metastatic breast cancer: a cost-effectiveness study.* Oncotarget, 2018. **9**(28): p. 19836-19846.
30. Venema, C., et al., *18F-FES PET Has Added Value in Staging and Therapy Decision Making in Patients With Disseminated Lobular Breast Cancer.* Clin Nucl Med, 2017. **42**(8): p. 612-614.
31. Jameera Begam, A., S. Jubie, and M.J. Nanjan, *Estrogen receptor agonists/antagonists in breast cancer therapy: A critical review.* Bioorg Chem, 2017. **71**: p. 257-274.
32. Gong, C., et al., *A preliminary study of (18)F-FES PET/CT in predicting metastatic breast cancer in patients receiving docetaxel or fulvestrant with docetaxel.* Sci Rep, 2017. **7**(1): p. 6584.
33. Chae, S.Y., et al., *A Randomized Feasibility Study of (18)F-Fluoroestradiol PET to Predict Pathologic Response to Neoadjuvant Therapy in Estrogen Receptor-Rich Postmenopausal Breast Cancer.* J Nucl Med, 2017. **58**(4): p. 563-568.

34. Liao, G.J., et al., *18F-Fluoroestradiol PET: Current Status and Potential Future Clinical Applications.* J Nucl Med, 2016. **57**(8): p. 1269-75.
35. Evangelista, L., V. Guarneri, and P.F. Conte, *18F-Fluoroestradiol Positron Emission Tomography in Breast Cancer Patients: Systematic Review of the Literature & Meta-Analysis.* Curr Radiopharm, 2016. **9**(3): p. 244-257.
36. van Kruchten, M., et al., *Positron emission tomography of tumour [(18)F]fluoroestradiol uptake in patients with acquired hormone-resistant metastatic breast cancer prior to oestradiol therapy.* Eur J Nucl Med Mol Imaging, 2015. **42**(11): p. 1674-1681.
37. Koleva-Kolarova, R.G., et al., *The value of PET/CT with FES or FDG tracers in metastatic breast cancer: a computer simulation study in ER-positive patients.* Br J Cancer, 2015. **112**(10): p. 1617-25.
38. Peterson, L.M., et al., *A phase 2 study of 16alpha-[18F]-fluoro-17beta-estradiol positron emission tomography (FES-PET) as a marker of hormone sensitivity in metastatic breast cancer (MBC).* Mol Imaging Biol, 2014. **16**(3): p. 431-40.
39. van Kruchten, M., et al., *PET imaging of oestrogen receptors in patients with breast cancer.* The Lancet Oncology, 2013. **14**(11): p. e465-e475.
40. Gemignani, M.L., et al., *Feasibility and predictability of perioperative PET and estrogen receptor ligand in patients with invasive breast cancer.* J Nucl Med, 2013. **54**(10): p. 1697-702.
41. van Kruchten, M., et al., *PET imaging of estrogen receptors as a diagnostic tool for breast cancer patients presenting with a clinical dilemma.* J Nucl Med, 2012. **53**(2): p. 182-90.
42. Dehdashti, F., et al., *PET-based estradiol challenge as a predictive biomarker of response to endocrine therapy in women with estrogen-receptor-positive breast cancer.* Breast Cancer Res Treat, 2009. **113**(3): p. 509-17.
43. Peterson, L.M., et al., *Quantitative imaging of estrogen receptor expression in breast cancer with PET and 18F-fluoroestradiol.* J Nucl Med, 2008. **49**(3): p. 367-74.
44. Linden, H.M., et al., *Quantitative fluoroestradiol positron emission tomography imaging predicts response to endocrine treatment in breast cancer.* J Clin Oncol, 2006. **24**(18): p. 2793-9.
45. Mortimer, J.E., et al., *Positron emission tomography with 2-[18F]Fluoro-2-deoxy-D-glucose and 16alpha-[18F]fluoro-17beta-estradiol in breast cancer: correlation with estrogen receptor status and response to systemic therapy.* Clin Cancer Res, 1996. **2**(6): p. 933-9.
46. Dehdashti, F., et al., *Positron tomographic assessment of estrogen receptors in breast cancer: comparison with FDG-PET and in vitro receptor assays.* J Nucl Med, 1995. **36**(10): p. 1766-74.
47. McGuire, A.H., et al., *Positron tomographic assessment of 16 alpha-[18F] fluoro-17 beta-estradiol uptake in metastatic breast carcinoma.* J Nucl Med, 1991. **32**(8): p. 1526-31.
48. Liu, C., et al., *Dual Tracers of 16alpha-[18F]fluoro-17beta-Estradiol and [18F]fluorodeoxyglucose for Prediction of Progression-Free Survival After Fulvestrant Therapy in Patients With HR+/HER2- Metastatic Breast Cancer.* Front Oncol, 2020. **10**: p. 580277.

49. Kurland, B.F., et al., *Between-patient and within-patient (site-to-site) variability in estrogen receptor binding, measured in vivo by 18F-fluoroestradiol PET.* J Nucl Med, 2011. **52**(10): p. 1541-9.
50. Yang, Z., et al., *Can positron emission tomography/computed tomography with the dual tracers fluorine-18 fluoroestradiol and fluorodeoxyglucose predict neoadjuvant chemotherapy response of breast cancer?--A pilot study.* PLoS One, 2013. **8**(10): p. e78192.
51. Barzaman, K., et al., *Breast cancer: Biology, biomarkers, and treatments.* Int Immunopharmacol, 2020. **84**: p. 106535.
52. Brooks, M., *Breast cancer screening and biomarkers.* Methods Mol Biol, 2009. **472**: p. 307-21.
53. Bettegowda, C., et al., *Detection of circulating tumor DNA in early- and late-stage human malignancies.* Sci Transl Med, 2014. **6**(224): p. 224ra24.
54. Dawson, S.J., et al., *Analysis of circulating tumor DNA to monitor metastatic breast cancer.* N Engl J Med, 2013. **368**(13): p. 1199-209.
55. Lanman, R.B., et al., *Analytical and Clinical Validation of a Digital Sequencing Panel for Quantitative, Highly Accurate Evaluation of Cell-Free Circulating Tumor DNA.* PLoS One, 2015. **10**(10): p. e0140712.
56. Chen, S. and Y. Liu, *p53 involvement in clonal hematopoiesis of indeterminate potential.* Curr Opin Hematol, 2019. **26**(4): p. 235-240.
57. Shivapurkar, N. and A.F. Gazdar, *DNA methylation based biomarkers in non-invasive cancer screening.* Curr Mol Med, 2010. **10**(2): p. 123-32.
58. Varley, K.E., et al., *Dynamic DNA methylation across diverse human cell lines and tissues.* Genome Res, 2013. **23**(3): p. 555-67.
59. Varley, K.E. and R.D. Mitra, *Nested Patch PCR for highly multiplexed amplification of genomic loci.* Cold Spring Harb Protoc, 2009. **2009**(7): p. pdb.prot5252.
60. Liu, M.C., et al., *Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA.* Annals of Oncology, 2020. **31**(6): p. 745-759.
61. Cohen, J.D., et al., *Detection and localization of surgically resectable cancers with a multi-analyte blood test.* Science, 2018. **359**(6378): p. 926-930.
62. Phallen, J., et al., *Direct detection of early-stage cancers using circulating tumor DNA.* Sci Transl Med, 2017. **9**(403).
63. Garcia-Murillas, I., et al., *Assessment of Molecular Relapse Detection in Early-Stage Breast Cancer.* JAMA oncology, 2019. **5**(10): p. 1473-1478.
64. Coombes, R.C., et al., *Personalized Detection of Circulating Tumor DNA Antedates Breast Cancer Metastatic Recurrence.* Clin Cancer Res, 2019. **25**(14): p. 4255-4263.
65. Li, W., et al., *CancerDetector: ultrasensitive and non-invasive cancer detection at the resolution of individual reads using cell-free DNA methylation sequencing data.* Nucleic Acids Res, 2018. **46**(15): p. e89.
66. Yang, X., L. Gao, and S. Zhang, *Comparative pan-cancer DNA methylation analysis reveals cancer common and specific patterns.* Brief Bioinform, 2017. **18**(5): p. 761-773.
67. Moran, S., et al., *Epigenetic profiling to classify cancer of unknown primary: a multicentre, retrospective analysis.* Lancet Oncol, 2016. **17**(10): p. 1386-1395.
68. Kang, S., et al., *CancerLocator: non-invasive cancer diagnosis and tissue-of-origin prediction using methylation profiles of cell-free DNA.* Genome Biol, 2017. **18**(1): p. 53.

69. Narbe, U., et al., *The Distribution of Circulating Tumor Cells Is Different in Metastatic Lobular Compared to Ductal Carcinoma of the Breast-Long-Term Prognostic Significance.* Cells, 2020. **9**(7).
70. Pramod, N., et al., *Comprehensive Review of Molecular Mechanisms and Clinical Features of Invasive Lobular Cancer.* Oncologist, 2021. **26**(6): p. e943-e953.
71. White, C.M., et al., *The pharmacokinetics of intravenous estradiol--a preliminary study.* Pharmacotherapy, 1998. **18**(6): p. 1343-6.
72. DeVore, G.R., O. Owens, and N. Kase, *Use of intravenous Premarin in the treatment of dysfunctional uterine bleeding--a double-blind randomized control study.* Obstet Gynecol, 1982. **59**(3): p. 285-91.
73. Schulman, S.P., et al., *Effects of acute hormone therapy on recurrent ischemia in postmenopausal women with unstable angina.* J Am Coll Cardiol, 2002. **39**(2): p. 231-7.
74. Rosano, G.M., et al., *Beneficial effect of oestrogen on exercise-induced myocardial ischaemia in women with coronary artery disease.* Lancet, 1993. **342**(8864): p. 133-6.
75. Mejia, A.A., et al., *Estimation of absorbed doses in humans due to intravenous administration of fluorine-18-fluorodeoxyglucose in PET studies.* J Nucl Med, 1991. **32**(4): p. 699-706.
76. Mankoff, D.A., et al., *[18F]fluoroestradiol radiation dosimetry in human PET studies.* J Nucl Med, 2001. **42**(4): p. 679-84.
77. Venema, C.M., et al., *Recommendations and Technical Aspects of 16alpha-[18F]Fluoro-17beta-Estradiol PET to Image the Estrogen Receptor In Vivo: The Groningen Experience.* Clin Nucl Med, 2016. **41**(11): p. 844-851.
78. Dehdashti, F., et al., *Positron emission tomographic assessment of "metabolic flare" to predict response of metastatic breast cancer to antiestrogen therapy.* Eur J Nucl Med, 1999. **26**(1): p. 51-6.
79. Flanagan, F.L., F. Dehdashti, and B.A. Siegel, *PET in breast cancer.* Semin Nucl Med, 1998. **28**(4): p. 290-302.
80. Jonson, S.D., et al., *Comparative breast tumor imaging and comparative in vitro metabolism of 16alpha-[18F]fluoroestradiol-17beta and 16beta-[18F]fluoromoxestrol in isolated hepatocytes.* Nucl Med Biol, 1999. **26**(1): p. 123-30.
81. Katzenellenbogen, J.A., M.J. Welch, and F. Dehdashti, *The development of estrogen and progestin radiopharmaceuticals for imaging breast cancer.* Anticancer Res, 1997. **17**(3b): p. 1573-6.
82. Linden, H.M. and F. Dehdashti, *Novel methods and tracers for breast cancer imaging.* Semin Nucl Med, 2013. **43**(4): p. 324-9.
83. Mortimer, J.E., et al., *Metabolic Flare: Indicator of Hormone Responsiveness in Advanced Breast Cancer.* Journal of Clinical Oncology, 2001. **19**(11): p. 2797-2803.
84. Linden, H.M., et al., *Fluoroestradiol positron emission tomography reveals differences in pharmacodynamics of aromatase inhibitors, tamoxifen, and fulvestrant in patients with metastatic breast cancer.* Clin Cancer Res, 2011. **17**(14): p. 4799-805.
85. Mankoff, D.A., T.J. Tewson, and J.F. Eary, *Analysis of blood clearance and labeled metabolites for the estrogen receptor tracer [F-18]-16 alpha-fluoroestradiol (FES).* Nucl Med Biol, 1997. **24**(4): p. 341-8.
86. Peterson, L.M., et al., *Factors influencing the uptake of 18F-fluoroestradiol in patients with estrogen receptor positive breast cancer.* Nucl Med Biol, 2011. **38**(7): p. 969-78.

87. Peterson, L.M., et al., *Correction to: a Phase 2 Study of 16alpha-[18F]-Fluoro-17beta-Estradiol Positron Emission Tomography (FES-PET) as a Marker of Hormone Sensitivity in Metastatic Breast Cancer (MBC).* Mol Imaging Biol, 2019. **21**(1): p. 191.
88. Janmahasatian, S., et al., *Quantification of lean bodyweight.* Clin Pharmacokinet, 2005. **44**(10): p. 1051-65.

10. APPENDICES

APPENDIX A
Schedule of Events

APPENDIX B
[^{18}F]Fluoroestradiol Infusion Flow Chart for Staff

APPENDIX C
[^{18}F]Fluoroestradiol Patient Adverse Event Questionnaire

APPENDIX D
NCI Common Toxicity Criteria

APPENDIX A: Schedule of Events, Revised for Expansion Phase as of March 2022 Amendment

	Screening	Baseline FES-PET/CT Imaging Session	Optional Baseline FDG-PET/CT Imaging Session	Optional FES-PET/CT Imaging Session 4 weeks following treatment start
		[^{18}F]FES-PET/CT	**[^{18}F]FDG-PET/CT[5]**	**[^{18}F]FES-PET/CT**
Assessment/ Procedures				
Informed Consent	X			
Inclusion/ Exclusion Criteria	X			
Infusion of [^{18}F]Fluoroestradiol		X		X
[^{18}F]Fluoroestradiol PET/CT Imaging		X		X
Vital Signs[1]		X[1]		X[1]
Initial AE Assessment[2]		X		X
24-72 Hour AE Assessment[2]		X		X
Pregnancy Test		X[3]	X[3]	X[3]
[^{18}F]FDG-PET/CT Imaging			X[4]	
MethylPatch ctDNA assay[5]		X[5]		X[5]

(1) Includes: Heart Rate, Blood Pressure, and Temperature. Vitals signs will be collected prior to infusion and again at the completion of the imaging study. Height and weight will be recorded at the beginning of an imaging session.

(2) Adverse events collection will begin when a patient receives their dose of [^{18}F]FES and continue until 24 hours post injection. The [^{18}F]FES Patient Adverse Event Questionnaire (Appendix D) will be completed at the conclusion of the imaging study and again at approximately 24-72 hours post injection via telephone consultation.

(3) Patient must be postmenopausal, surgically sterile, or confirmed not to be pregnant by serum pregnancy test performed within 48 hours prior to research PET imaging.

(4) The optional [^{18}F]FDG-PET/CT session may occur before or after the [^{18}F]FES-PET/CT within 4 weeks before or after but may not occur on the same day.

(5) Requires one phlebotomy procedure and delivery of sample to HCI Biorepository and Molecular Pathology Shared Resource and subsequent MethylPatch PCR analysis by the HCI Varley Lab

Appendix A.2

MethylPatch ctDNA Survival Assessment Schedule

Assessment/ Procedures	Informed Consent	Inclusion/Exclusion Criteria	Optional MethylPatch ctDNA Assay	Assessment of Recurrence and Survival
Screening	X	X		
6-month follow-up (+/- 1 month)			X[1]	X[2]
12-month follow-up (+/- 1 month)			X[1]	X[2]
18-month follow-up (+/- 2 months)			X[1]	X[2]
24-month follow-up (+/- 2 months)			X[1]	X[2]
36-month follow-up (+/- 2 months)			X[1]	X[2]
48-month follow-up (+/- 2 months)			X[1]	X[2]
60-month follow-up (+/- 2 months)			X[1]	X[2]

1. Requires one phlebotomy procedure and delivery of sample to HCI Biorepository and Molecular Pathology Shared Resource and subsequent MethylPatch PCR analysis by the HCI Varley Lab
2. Survival assessment as based on electronic medical record clinical visit follow-up and participant phone call over a total 5-year time-to-event endpoint. Recurrence evaluation will include assessment of recurrent and/or metastatic disease in participants without evidence of distant metastatic disease at baseline assessment.

APPENDIX B: [^{18}F]Fluoroestradiol Infusion Flow Chart for Staff

Subject Name and MRN: _____

Subject Study ID (if enrolled): _____

Projected Study Start Date: _____ Referring MD: _____

The infusion and imaging procedure will be terminated in any patient who exhibits anaphylaxis, significant hypotension (systolic blood pressure less than 80 mmHg, dyspnea, chest pain, grand mal seizure or an O_2 saturation lower than 80%.

Administered dose of [^{18}F]FES _____ mCi

Specific Activity of [^{18}F]FES _____Ci/mmol

Initials of Person Making Assessment	Time	Study	Temp	BP	HR	Weight	Height
		Baseline					
		End of Study				NA	NA

Completed by: _____ Date: _____

APPENDIX C: [^{18}F]Fluoroestradiol Patient Adverse Event Questionnaire

Subject Name and MRN: _____

Subject Study ID (if enrolled): _____

Projected Study Start Date: _____ Referring MD: _____

Day of Imaging Session ☐

Possible Adverse Event	Yes	No	Comment on possible AE
Body as a Whole:			
Pain (Abdominal)			
Pain (Chest/Breast)			
Pain (Other site)			
Fever			
Injection site reaction			
Cardiovascular System:			
Vasodilation (flushing)			
Tachycardia (fast heart rate)			
Digestive System:			
Nausea			
Diarrhea			
Vomiting			
Respiratory System:			
Dyspnea (Shortness of breath)			
Skin and Appendages:			
Rash			
Pruritus (Itching)			
Urticaria (Hives)			
Sweating			
Cyanosis (discoloration of fingers/toes)			
Central Nervous System			
Visual disturbances			
Numbness of feet			
Numbness of fingers/hands			
Weakness of feet			
Weakness of fingers			
Burning sensation in feet			
Burning sensation of fingers			

Performed by: _____ Date: _____Time: _____

PI Oversight: _____ Date: _____

24-72 hour post-injection assessment ☐

Possible Adverse Event	**Yes**	**No**	**Comment on possible AE**
Body as a Whole:			
Pain (Abdominal)			
Pain (Chest/Breast)			
Pain (Other site)			
Fever			
Injection site reaction			
Cardiovascular System:			
Vasodilation (flushing)			
Tachycardia (fast heart rate)			
Digestive System:			
Nausea			
Diarrhea			
Vomiting			
Respiratory System:			
Dyspnea (Shortness of breath)			
Skin and Appendages:			
Rash			
Pruritus (Itching)			
Urticaria (Hives)			
Sweating			
Cyanosis (discoloration of fingers/toes)			
Central Nervous System			
Visual disturbances			
Numbness of feet			
Numbness of fingers/hands			
Weakness of feet			
Weakness of fingers			
Burning sensation in feet			
Burning sensation of fingers			

Performed by: _____ Date: _____Time: _____

PI Oversight: _____ Date: _____

APPENDIX D: 5 Year Follow-up Assessment

Subject Name and MRN: _____

Subject Study ID (if enrolled): _____

Assessment Timepoint	Is the participant alive?	Imaging performed at our institution since prior assessment?	Imaging performed at outside institution since prior assessment?	Clinical evaluation at our institution since prior assessment?	Clinical evaluation at outside institution since prior assessment?	Is there documented recurrent or new metastatic disease based on these assessments?
6-month follow-up (+/- 1 month)						
12-month follow-up (+/- 1 month)						
18-month follow-up (+/- 2 months)						
24-month follow-up (+/- 2 months)						
36-month follow-up (+/- 2 months)						
48-month follow-up (+/- 2 months)						
60-month follow-up (+/- 2 months)						

Instructions: Mark yes, no, unable to confirm for each of the questions above. Any imaging results or pertinent clinical records from the clinical providers monitoring the patient's breast cancer therapy (as determined by the study team/PI) will be obtained and uploaded into the research record.

APPENDIX E: NCI Common Toxicity Criteria

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

[i] The United States Pharmacopeia. The National Formulary. General Official Monograph: Fludeoxyglucose F 18 Injection. Official from May 1, 2007;USP 30 NF 25 Vol. 2:2158-2159. The United States Pharmacopeial Convention.