Hybrid Molecular Imaging of Estrogen Receptor in Breast Cancer Patients with Ductal Carcinoma In Situ

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ABBREVIATIONS

AE	Adverse event
AUC	Area under the curve
CAEPR	Comprehensive Adverse Events and Potential Risks
CFR	Code of federal regulation
cGMP	current Good Manufacturing Practice
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CIP	Cancer Imaging Program
cm	Centimeter
COV	Coefficient of variation
CT	Computed tomography
DCE-MRI	Dynamic contrast enhanced magnetic resonance imaging
DCIS	Ductal carcinoma in situ
DICOM	Digital imaging and communications in medicine
DOT	Disease Oriented Team
DSMC	Data and Safety Monitoring Committee
DWI	Diffusion weighted imaging
ECOG/ACRIN	Eastern Cooperative Oncology Group/American College of Radiology Imaging Network
ERα+	Estrogen receptor alpha-positive
FDA	U.S. Food and Drug Administration
FDG	^{18}F-fluorodeoxyglucose
FES	^{18}F-fluoroestradiol
GCP	Good Clinical Practice
GE	General Electric
H&E	hematoxylin and eosin
HER2	Human epidermal growth factor receptor 2
HIPAA	Health Insurance Portability and Accountability Act
HPLC	High performance liquid chromatography
ICC	Intra-class correlation coefficient
IHC	Immunohistochemistry
IME	Important medical event
IND	Investigational new drug
IRB	Institutional review board
IV	Intravenous
Kg	Killogram
M	molar
MBq	MegaBecquerel
mCi	MilliCurie
mGy	MilliGray
mL	Milliliter
mmol	millimole
mrem	milli Roentgen equivalent man
MRI	Magnetic resonance imaging

MRN	Medical record number
MR/PET	Magnetic resonance/Positron emission tomography
mSv	MilliSievert
MTV	Metabolic tumor volume
NCI	National Cancer Institute
oz	ounce
PACS	Picture archiving and communication system
PET	Positron emission tomography
PET/MRI	Positron Emission Tomography/Magnetic Resonance Imaging
PSR	Protocol summary report
PI	Principal investigator
PR	Progesterone receptor
QA	Quality Assurance
QC	Quality Control
ROC	Receiver operator curve
ROI	Region of interest
SAE	Serious adverse event
SBP/SHBG	Sex steroid-binding protein/Sex hormone binding globulin
SUV	Standard uptake value
TLU	Total lesion uptake
TRIP	Translational Research Initiatives in Pathology
USP	United States Pharmacopeia
UW	University of Wisconsin
UWCCC	University of Wisconsin Carbone Cancer Center

1. PROTOCOL SUMMARY

TITLE OF STUDY
Hybrid Molecular Imaging of Estrogen Receptor in Breast Cancer Patients with Ductal Carcinoma In Situ

CLINICAL PHASE
Phase 2

INVESTIGATORS
Lead clinical investigator: Amy Fowler, MD, PhD, University of Wisconsin-Madison
Sub-clinical investigator: Roberta Strigel, MD, MS, University of Wisconsin-Madison

This is an investigator-initiated clinical trial performed at a single clinical site.

PERIOD OF TRIAL
Planned study conduct duration of approximately 3 years.

STUDY OBJECTIVES
Integrated whole-body magnetic resonance imaging (MRI)-positron emission tomography (PET) scanners have recently been introduced for clinical use. This technology combines the anatomic and perfusion data obtained with Dynamic Contrast Enhanced (DCE) MRI with functional imaging data obtained from PET. For breast imaging, the combination of MRI and PET has important potential to improve diagnostic accuracy and provide molecular characterization of breast cancer. The overall purpose of this research is to determine the technical feasibility of simultaneous breast DCE MRI with ^{18}F-FES PET for measuring estrogen receptor (ER) in patients with ductal carcinoma in situ (DCIS) and identifying patients with low-risk of disease recurrence. We hypothesize that quantitative ^{18}F-FES uptake parameters from PET/MRI will correlate well with the ER immunohistochemistry score and with low-risk recurrence scores.

Primary Objective

To compare quantitative ^{18}F-FES uptake of biopsy-proven DCIS measured using PET/MRI with ER protein levels determined by immunohistochemistry.

Secondary Objectives

1) To determine the optimal cut-point ^{18}F-FES uptake value for distinguishing between ER+ and ER-negative DCIS; 2) to determine the test-retest reproducibility of quantitative assessment of tumor ^{18}F-FES uptake; 3) to determine the optimal cut-point ^{18}F-FES uptake value for distinguishing between low-risk DCIS and intermediate/high-risk DCIS; 4) to estimate the association of quantitative ^{18}F-FES uptake (continuous SUV_{max}) with research-based Oncotype DX DCIS scores (0-100); 5) to measure the upgrade rate to invasive cancer at surgical excision; 6) to correlate tumor ^{18}F-FES uptake with serum estradiol and sex hormone binding globulin levels.

Exploratory Objective

To correlate tumor cell density with ^{18}F-FES uptake on PET/MRI.

INCLUSION CRITERIA

- Women 18 years of age or older
- Diagnosis of biopsy-proven DCIS without invasion or microinvasion measuring at least 1.0 cm in diameter by any imaging modality
- Undergoing diagnostic breast MRI ordered by the referring clinician for staging and extent of disease*

EXCLUSION CRITERIA

- Inability or unwillingness to provide informed consent to the study
- Surgery, radiation, neoadjuvant chemo/endocrine therapy for the current malignancy prior to study enrollment
- Patients currently taking or have taken an ER-blocking medication (e.g. tamoxifen, raloxifene) within 6 weeks prior to study enrollment
- Pregnant or lactating women
- Patient with intolerance or contraindications for MRI or gadolinium-based contrast agents
- Patient girth exceeds the bore of the MRI/PET scanner
- Patients with a history of allergic reaction attributable to compounds of similar chemical or biologic composition to ^{18}F-FES
- Patients in liver failure as judged by the patient's physician, due to the hepatobiliary clearance of ^{18}F-FES
- Patients requiring intravenous (IV) conscious sedation for imaging are not eligible; patients requiring mild, oral anxiolytics for the clinical MRI will be allowed to participate as long as the following criteria are met:
    - The subject has their own prescription for the medication
    - The informed consent process is conducted prior to the self-administration of this medication
    - They come to the research visit with a driver or an alternative plan for transportation (e.g. Uber, taxi, etc.)

STUDY DESIGN

This is a prospective, one-arm, observational study which will enroll patients with biopsy-proven DCIS scheduled for diagnostic breast MRI for preoperative staging/extent of disease evaluation as part of standard of care. Eligible patients will be consented for participation in the research study which includes a directed breast PET/MRI with ^{18}F-FES. ^{18}F-FES uptake of the known malignancy will be measured on the PET/MRI examination using standardized uptake values (SUV) and tumor-to-normal tissue ratios. The proposed work-flow is described in the schema.

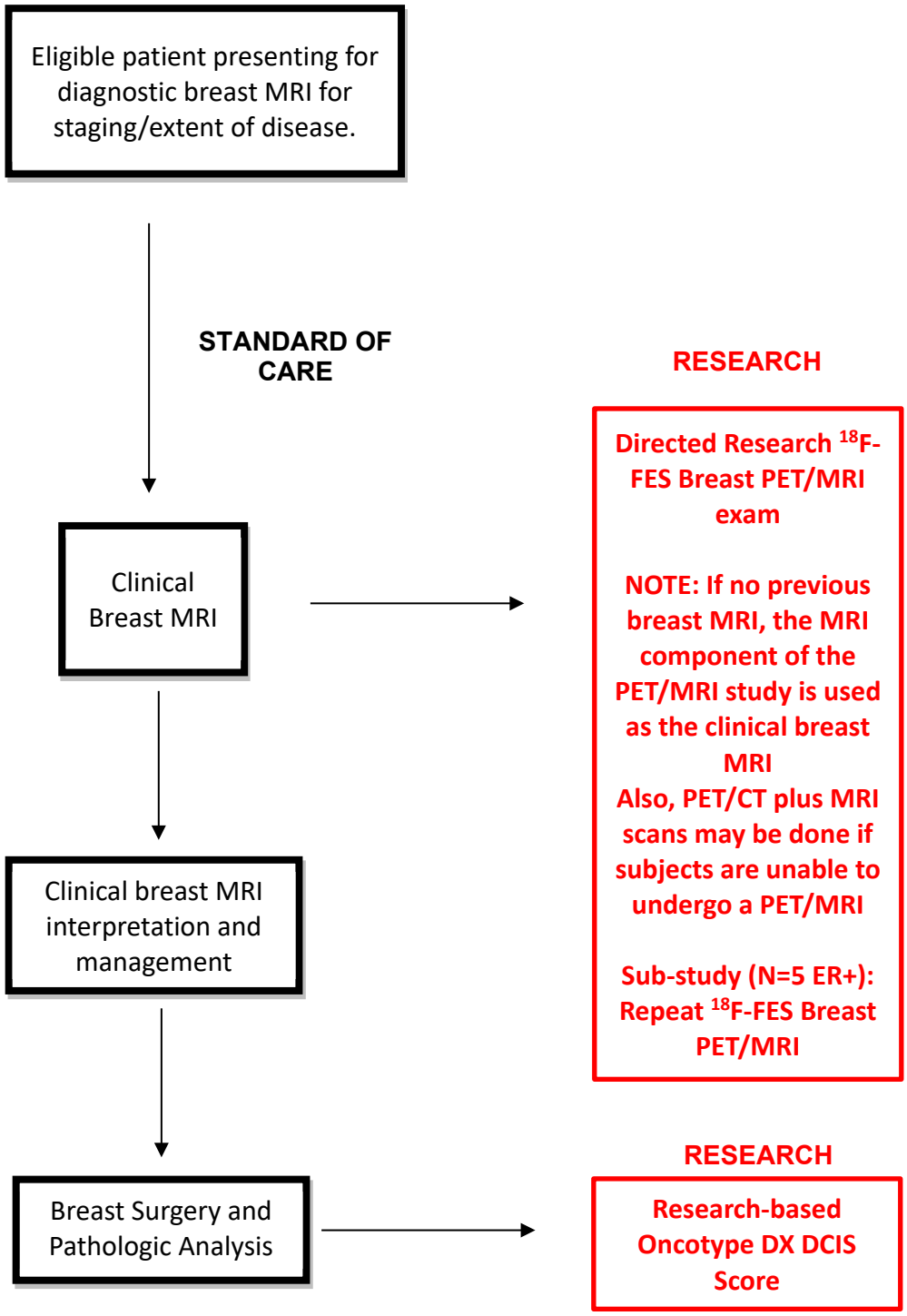
* Patients with newly diagnosed DCIS from a screening breast MRI are also eligible to participate in this study. For these subjects, the research breast PET/MRI should be performed prior to any treatment or surgery for DCIS.

NUMBER OF SUBJECTS

We will test the hypothesis that ^{18}F-FES SUV_{max} from PET/MRI correlates well against the ER IHC Allred score. The correlation of the two measures will be evaluated using Spearman correlation analysis. A sample size of 12 patients achieves 82% power (β=0.20) for detecting an expected correlation coefficient (r=0.73) to be significantly different from zero with two-tailed α value of 0.05.

We anticipate enrolling approximately 1 subject every 1-2 months . To measure test-retest reproducibility, a subset of up to 5 participants with ER+ DCIS will have the option to for both test-retest FES scans. The number of subjects with ER-negative DCIS will be limited to ≤3.

SCHEMA


Eligible patient presenting for diagnostic breast MRI for staging/extent of disease.
STANDARD OF CARE
Clinical Breast MRI
Clinical breast MRI interpretation and management
Breast Surgery and Pathologic Analysis
RESEARCH
Directed Research 18F-FES Breast PET/MRI exam
NOTE: If no previous breast MRI, the MRI component of the PET/MRI study is used as the clinical breast MRI
Also, PET/CT plus MRI scans may be done if subjects are unable to undergo a PET/MRI
Sub-study (N=5 ER+): Repeat 18F-FES Breast PET/MRI
RESEARCH
Research-based Oncotype DX DCIS Score

2. INTRODUCTION

DCIS comprises up to one-fourth of all newly diagnosed cases of breast cancer (1). The incidence of DCIS has markedly increased since the widespread utilization of screening mammography. It is estimated that approximately 63,410 new cases of in situ carcinoma will be diagnosed in the United States in 2017 (2). Despite the high prevalence of DCIS, overall treatment outcomes are excellent with 3.3% breast-cancer specific mortality after 20 years (3).

DCIS is a non-obligate precursor to invasive breast cancer, meaning some disease will remain DCIS if not surgically excised while others will progress to invasive cancer (4, 5). Small study series of the natural history of DCIS have found that approximately 28% (7/25) of untreated DCIS progressed to invasive disease within 15 years (6). Even cases of low nuclear grade DCIS have been shown to develop into invasive breast cancer in 36% (16/45) of patients undergoing biopsy only (7). In contrast, data also exist from autopsy studies that have found undiagnosed DCIS in up to 15% of women who died from causes other than breast cancer (8).

The conventional treatment of DCIS is surgical excision, either through lumpectomy or mastectomy (9). For patients eligible for breast conserving surgery, radiation therapy is typically recommended after lumpectomy (10) since adjuvant radiotherapy has been shown to reduce the risk of local recurrence (either recurrent DCIS or invasive cancer) by approximately 50% (11). Compilation of data from four randomized trials consisting of 3,729 women with DCIS have shown that radiation therapy reduced the ten-year rate of local recurrence from 28.1% in patients randomized to surgery alone versus 12.9% for those randomized to surgery with radiation therapy (11). Adjuvant endocrine therapy (tamoxifen or aromatase inhibitors) can also be considered as an additional systemic risk-reducing treatment option in women with estrogen receptor (ER) positive DCIS (12-16).

In contrast with invasive breast cancer, use of adjuvant radiotherapy or endocrine therapy for DCIS patients has not yet been shown to improve survival (11, 12). Thus, the potential for over-diagnosis and over-treatment of DCIS has become a controversial topic. Improved individualized treatment plans for women with DCIS and prevention of over-treatment in patients with “low-risk” DCIS has been ranked highly as a critical and impactful research priority (17-20). There are ongoing clinical trials evaluating the comparative effectiveness of treatments that are alternatives to the standard described above. These include the E4112 trial, which seeks to avoid radiation therapy in women with low risk DCIS and the COMET trial which is evaluating endocrine therapy as an alternative local control approach for women with ER/PR+ low and intermediate grade pure DCIS.

Current approaches for risk stratification are primarily based on clinical and pathological factors. These include patient age, nuclear grade, size, and surgical margin status. Prognostic models, such as the University of Southern California/Van Nuys Prognostic Index (21) and the Memorial Sloan-Kettering Cancer Center Nomogram (22), have been developed that incorporate these clinicopathologic variables. These models are available to clinicians to provide a patient with information regarding the probability of local

recurrence with and without adjuvant therapy to facilitate shared decision-making. A drawback of this approach is that the methods have not been fully validated using independent data sets outside of the institutions from which they were developed (23, 24).

A multigene expression assay has been recently developed that provides a ten-year risk estimate of local recurrence for DCIS patients following breast conserving therapy alone (25). The 12-gene Oncotype DX DCIS Score is generated from expression levels of seven cancer related genes and five reference genes determined from a patient's biopsy or surgical specimen (25). The score is a continuous variable from 0 to 100. Low-risk scores are 0 to 38. Intermediate-risk scores are 39 to 54. High-risk scores are 55 to 100. The score was initially validated using data from the Eastern Cooperative Oncology Group (ECOG) E5194 clinical trial, a prospective cohort study of 327 patients deemed to have low-risk of local recurrence based on clinicopathologic features treated with breast conserving surgery alone. The 10-year risks (95%CI) of any ipsilateral breast event (local recurrence of DCIS or invasive carcinoma) for low, intermediate, and high risk scores were 10.6% (6.9-16.2%), 26.7% (16.2-41.9%) and 25.9% (14.8-43%), respectively and 3.7% (1.8-7.7%), 12.3% (5.1-27.8%), and 19.2% (9.5-36.4%), respectively, for an invasive ipsilateral breast event (25). The DCIS score was further validated in a more diverse patient population treated with breast conserving surgery alone, the Ontario DCIS cohort (26, 27). The 10-year risks (95% CI) of any ipsilateral breast event for these 571 patients with low, intermediate, and high risk scores were 12.7% (9.5-16.9%), 33.0% (23.6-44.8%), and 27.8% (20.0-37.8%), respectively (26). Thus, this genomic test has been shown to provide information on local recurrence independent of traditional clinicopathologic factors such as age, size, and grade (25, 26) and has been shown to have clinical utility in influencing patient management (28, 29). However, the test is expensive (~$3500) and may not be cost effective (30). Furthermore, it requires a 3-week turnaround time (29) and sufficient tissue for analysis which may introduce sampling bias.

An important molecular target in premalignant and malignant breast tissue is ER, which drives hormone-dependent breast tumor growth, and can be inhibited by endocrine therapy. As shown in invasive breast cancer, ER appears to be an important prognostic factor and predictive biomarker for clinical benefit of adjuvant tamoxifen treatment for DCIS (16). In normal breast tissue, ER is expressed in a minority of cells at low to moderate staining intensity. However, very high levels of ER expression in nearly all cells are noted in most premalignant and noninvasive breast lesions (e.g. hyperplasias, atypia, lobular carcinoma in situ, and non-comedo DCIS) (31). The majority of DCIS express ER protein (approximately 69%; range 49-97%) (32). The exception is high grade comedo DCIS which can have low or no ER expression. Semi-quantitative ER levels measured by immunohistochemistry in DCIS are inversely related to the histologic grade of DCIS and are increased compared to invasive breast cancer (33). Interestingly, tumors cells at sites of focally disrupted myoepithelial cell layers are predominately (86.4%) ER-negative (34).

Expression of ER can be measured in patients through the use of a radiolabeled estrogen ligand, 16α-^{18}F-fluoro-17β-estradiol (^{18}F-FES), and positron emission tomography (PET)

imaging (35, 36). Tumoral ^{18}F-FES uptake, as measured by SUV_{max} with whole body PET/CT imaging, correlates well (r=0.56-0.96) with ER expression in invasive breast cancer, as measured by radioligand binding in fresh tissue and immunohistochemistry in fixed tissue (37, 38). Overall sensitivity of ^{18}F-FES PET for detection of ER+ breast cancer was 84% (95% CI: 73–91) in four published studies involving 114 patients (35, 37-40). Overall specificity was high at 98% (95% CI: 90–100) demonstrated by absence of ^{18}F-FES uptake (SUV_{max} less than 1.0) in 51 of 52 histologically benign lesions and ER-negative breast cancer in three studies (35, 38-40).

^{18}F-FES PET imaging has been studied as a predictive factor for clinical benefit of endocrine therapy in patients with metastatic ER+ breast cancer (35, 36). Van Kruchten *et al.* combined the results of four single-institution studies and determined that lack of response to endocrine therapy was predicted by ^{18}F-FES SUV_{max} under 1.5 in this heterogeneous group of ER+ patients (35). Using the 1.5 SUV_{max} threshold, 96 of 114 patients would have been selected to receive endocrine therapy, and 62 of these would have had a clinical benefit (PPV of 65%). Alternatively, of the 42 patients with ^{18}F-FES SUV below 1.5, 37 derived no clinical benefit from endocrine therapy (NPV 88%).

The addition of magnetic resonance imaging (MRI) provides exquisite anatomic detail to characterize tumor morphologic characteristics and for functional assessment of lesion perfusion (41). In addition to anatomic data, certain advanced MRI techniques, such as diffusion weighted imaging, has potential to distinguish low versus high risk DCIS (42, 43). The ECOG/ACRIN trial E4112 titled “Prospective Study of Magnetic Resonance Imaging (MRI) and Multiparameter Gene Expression Assay in Ductal Carcinoma In Situ (DCIS)” is a multi-institutional study of 368 patients and includes a secondary outcome measure assessing the relation between MRI morphologic and kinetic features and DCIS score.

To the best of our knowledge, no studies have been performed using ^{18}F-FES in patients with DCIS. This study will investigate the technical feasibility of simultaneous breast DCE MRI with ^{18}F-FES PET for quantifying ER in patients with DCIS and identifying patients with low-risk of disease recurrence. If successful, we will pursue further studies using this imaging approach for stratifying patients with ER+ DCIS who may avoid radiation therapy (as in the E4112 study) and/or surgery (as in the COMET trial) and potentially derive the most benefit from endocrine therapy.

3. STUDY AIMS/STUDY OBJECTIVES

The goal of this research is to assess the technical feasibility of simultaneous breast DCE MRI with ^{18}F-FES PET for measuring ER in patients with DCIS and identifying patients with low-risk of disease recurrence. We hypothesize that quantitative ^{18}F-FES uptake parameters from PET/MRI will correlate well with the ER immunohistochemistry score and with low-risk recurrence scores.

3.1 Primary objective:

To measure ^{18}F-FES uptake of biopsy-proven DCIS using breast PET/MRI. The reference standard will be ER expression measured with immunohistochemistry.

3.2 Secondary objectives:

- Determine the optimal cut-point ^{18}F-FES uptake value for distinguishing between ER+ and ER-negative DCIS.
- Determine the test-retest reproducibility of quantitative assessment of tumor ^{18}F-FES uptake.
- Determine the optimal cut-point ^{18}F-FES uptake value for distinguishing between low-risk DCIS and intermediate/high-risk DCIS.
- Estimate the association of quantitative ^{18}F-FES uptake (continuous SUV_{max}) with research-based Oncotype DX DCIS scores (0-100)
- Measure the upgrade rate to invasive cancer at surgical excision.
- To correlate tumor ^{18}F-FES uptake with serum estradiol and sex hormone binding globulin levels.

3.3 Exploratory objective:
To correlate tumor cell density with ^{18}F-FES uptake on PET/MRI.

Study duration: 3 years. Our estimated rate of accrual is approximately 1 participant every 1-2 months.

4. SELECTION OF PATIENTS

Study Population:
Female patients of all races and ethnic backgrounds at least 18 years of age with a current diagnosis of DCIS who are undergoing breast MRI for staging and extent of disease ordered by their provider as part of routine clinical care.

4.1 Eligibility Criteria

Inclusion Criteria:

4.1.1 Women 18 years of age or older

4.1.2 Diagnosis of biopsy-proven DCIS without invasion or microinvasion measuring at least 1.0 cm in diameter by any imaging modality

4.1.3 Undergoing diagnostic breast MRI ordered by the referring clinician for staging and extent of disease[†]

Exclusion Criteria:

4.1.4 Inability or unwillingness to provide informed consent to the study

[†] Patients with newly diagnosed DCIS from a screening breast MRI are also eligible to participate in this study. For these subjects, the research breast PET/MRI should be performed prior to any treatment or surgery for DCIS.

4.1.5 Surgery, radiation, neoadjuvant chemo/endocrine therapy for the current malignancy prior to study enrollment
4.1.6 Patients currently taking or have taken an ER-blocking medication (e.g. tamoxifen, raloxifene) within 6 weeks prior to study enrollment
4.1.7 Pregnant or lactating women
4.1.8 Patient with intolerance or contraindications for MRI or gadolinium-based contrast agents
4.1.9 Patient girth exceeds the bore of the MRI/PET scanner
4.1.10 Patients with a history of allergic reaction attributable to compounds of similar chemical or biologic composition to FES
4.1.11 Patients in liver failure as judged by the patient's physician, due to the hepatobiliary clearance of ^{18}F-FES

4.1.12 Patients requiring intravenous (IV) conscious sedation for imaging are not eligible; patients requiring mild, oral anxiolytics for the clinical MRI scan will be allowed to participate as long as the following criteria are met:

4.1.12.1 The patient has their own prescription for the medication

4.1.12.2 The informed consent process is conducted prior to the self-administration of the medication.

4.1.12.3 The patient comes to the research visit with a driver or an alternative plan for transportation (e.g. Uber, taxi, etc.).

5. RESEARCH DESIGN AND METHODS

5.1 Study Design

This prospective, single-arm, observational, single-institutional research study will enroll women with DCIS scheduled for diagnostic breast MRI for preoperative staging/extent of disease evaluation. The proposed work-flow is described in the schema above. Eligible patients will be consented for participation in the research study which will involve the simultaneous acquisition of ^{18}F-FES PET imaging data during a standard dynamic contrast enhanced clinical breast MRI performed prior to surgical excision. Patients with ER+ DCIS will be offered to participate in an optional sub-study which will involve a second (test-retest) ^{18}F-FES PET/MRI examination until we have accrued up to 5 patients. The results of the ^{18}F-FES PET imaging component of the examination will not be used to guide patient management. However, the clinical breast MRI component of the examination will be interpreted and reported with management recommendation as per routine clinical care. Surgical management, radiation therapy, and adjuvant therapy will follow standard of care. A research-based Oncotype DX DCIS score will be obtained from the surgical specimen for research purposes only since this test is currently not frequently used clinically at our institution (only about 1-5% DCIS patients locally).

Patients with DCIS may also participate in this study if a clinical breast MRI has already been performed. In this scenario, a research ^{18}F-FES breast PET/MRI will be performed on a separate day as the clinical breast MRI exam prior to surgery.

The primary objective is to measure ^{18}F-FES uptake of biopsy-proven DCIS using PET/MRI. The reference standard will be ER expression measured with immunohistochemistry. Secondary objectives include 1) to determine the optimal cut-point ^{18}F-FES uptake value for distinguishing between ER+ and ER-negative DCIS; 2) to determine the test-retest reproducibility of quantitative assessment of tumor ^{18}F-FES uptake; 3) to determine the optimal cut-point ^{18}F-FES uptake value for distinguishing between low-risk DCIS and intermediate/high-risk DCIS; 4) to estimate the association of quantitative ^{18}F-FES uptake (continuous SUV_{max}) with research-based Oncotype DX DCIS scores (0-100); 5) to measure the upgrade rate to invasive cancer at surgical excision; 6) to correlate tumor ^{18}F-FES uptake with serum estradiol and sex hormone binding globulin levels. An exploratory objective is to correlate tumor cell density with ^{18}F-FES uptake on PET/MRI.

5.2 Reference Standard for ER Expression

The primary objective is to measure ^{18}F-FES uptake of biopsy-proven DCIS using PET/MRI. The reference standard will be ER expression measured with immunohistochemistry performed as per the current standard of care.

ER status is reported as positive or negative and includes a percentage of cells staining positive as well as intensity of staining. This information will be obtained from the clinical pathology report in the subjects' electronic medical record and will be used to calculate a semi-quantitative Allred score (44). If the information needed to calculate the Allred score is not included in the clinical pathology report, a pathologist on the research study team will review the existing ER IHC slides.

The Allred score is the sum of the proportion score and the intensity score (**Table 1**) (44). ER-negative cancers can have either an Allred score of 0 or 2 (1+1). ER-positive cancers can have an Allred score of 3, 4, 5, 6, 7, or 8. An Allred score of 1 does not exist.

Table 1: Allred Scoring System

Percentage of Positive-Staining Tumor Cells	Proportion Score (PS)	Average Intensity of Positive Tumor Cells	Intensity Score (IS)
None	0	None	0
<1%	1	Weak	1
1-10%	2	Intermediate	2
10-33%	3	Strong	3
33-66%	4		
66-100%	5		

5.3 Study Calendar/Schedule

The study calendar/schedule is detailed in **Table 2**.

Table 2: Study Calendar/Schedule

Study Activity	Baseline[a]	Imaging Day[b]	Clinical Follow-Up[c]
Informed Consent[h]	X		
Demographics[d]	X		
Medical History[d]	X		
Radiology Reports[d]	X		X
Pathology Reports (Breast biopsy and final surgical excision)[d]	X		X
Concomitant Medication Review		X	
Pregnancy Screening		X	
Day of last menstrual period (if premenopausal)		X	
Height and Weight		X	
Blood for Hormone Levels		X[g]	
^{18}F-FES PET/MRI[i]		X	
Adverse Event Evaluation[e]		X	X
Research-based Oncotype DX DCIS Score[f]			X

(a) Baseline is defined as the time period from initial subject contact until injection of ^{18}F-FES.
(b) Imaging Day is defined as the day of the research ^{18}F-FES PET/MRI imaging session.
(c) Subjects' medical records are followed until final surgical excision and clinical determination of adjuvant therapy plan. Any standard of care labs, procedures, and/or imaging exams obtained during this time period will be reviewed. Data regarding recommended adjuvant therapy (radiation and endocrine therapy) will be recorded. There are no protocol requirements during this time frame.
(d) Clinical data, including age, race/ethnicity, primary breast cancer data (standard clinical histopathology including tumor biomarkers, location, size, and date of biopsy/diagnosis), mammographic breast density, and menopausal status will be recorded from the electronic medical record.
(e) Adverse events occurring within one day after ^{18}F-FES infusion will be recorded by the study coordinator who will contact the subject within 1 to 3 days.
(f) Oncotype DX DCIS score will be obtained from the electronic medical record if performed clinically (only 1-5% of DCIS patients locally expected) and will also be performed as a separate research-based assay using the resection specimen.
(g) Blood sample collected will be up to 10 mLs total.
(h) During the COVID-19 pandemic, consent procedures will be conducted by phone, when possible, to minimize face-to-face contact subjects have with the research team. An encrypted copy of the consent form will be mailed or emailed to subjects prior to the scheduled consent phone call.
(i) Subjects who are unable to undergo a PET/MRI scan will be invited to undergo a PET/CT along with a separate MRI scan

5.4 Clinical Breast MRI (Standard of Care)

The clinical breast MRI will be performed and interpreted according to standard clinical practice. If additional management recommendations are generated based on the clinical breast MRI (i.e. identification of unsuspected suspicious findings requiring biopsy), the research examinations will ideally be scheduled prior to the date of scheduled intervention(s) but can also be performed after a biopsy

procedure. The patient will be informed of this possibility at the time of initial consent.

5.5 Research Breast ^{18}F-FES PET/MRI

5.5.1 Timing of ^{18}F-FES PET/MRI

Patients must complete the research breast PET/MRI study prior to surgical/oncologic intervention for their biopsy-proven breast cancer as part of their routine clinical care. Ideally, the research breast PET/MRI study should be performed prior to any interventions (i.e. biopsies) prompted by the clinical breast MRI if performed prior to the research study, but this is not required. If patients are also scheduled for clinical nuclear medicine studies using ^{99m}Tc-based or ^{18}F-based radiopharmaceuticals, the research study should be scheduled on a separate day.

5.5.2 Patient Preparation

The patient does <u>not</u> need to be fasting for ^{18}F-FES PET/MRI. Prior to arrival, the patient should be well-hydrated (encourage drinking two-three 8-12 oz glasses of water) unless a fluid-restriction diet is prescribed by the patient's treating physician (e.g., congestive heart failure). The patient should continue to take any of their prescribed medications as scheduled by their treating physician.

Upon arrival, subject compliance with pre-procedure instructions will be confirmed. Subject completion of the informed consent documentation will also be confirmed. The patient's height and weight will be measured and recorded. Women with childbearing potential will be asked to confirm they are not pregnant. If they cannot confirm, they will have a urine pregnancy test either at the imaging study visit or within 7 days prior to the imaging study visit.

5.5.3 ^{18}F-FES Administration

The patient will have an intravenous line placed, typically in the hand or arm opposite to the known primary breast cancer, ^{18}F-FES will be given by 2-minute infusion, and the dose administered will be approximately 6 mCi ± 20%. The injection site will be inspected by the technologist to assess for any possible infiltration. The patient will wait approximately 60 minutes to allow for biodistribution and tumor uptake of the radiopharmaceutical prior to imaging. The patient should void prior to image acquisition.

All adverse events occurring within a 24-hour period post-infusion will be recorded (a phone call to the participant performed 1 to 3 days after ^{18}F-FES infusion is acceptable means of collecting this information). The adverse events to be specifically monitored during the infusion include localized discomfort at the IV injection site, pain, respiratory difficulties, flushing, dizziness, pruritus/rash, and any other symptoms that could be

secondary to an anaphylactic reaction. The subject will be instructed to report any subjective symptoms or sensory changes noted.

5.5.4 Imaging Quality Assurance (QA)/Quality Control (QC) Procedure
QA/QC procedures will include review of DICOM files against study protocols. The PET/MRI scanner must be kept calibrated in accordance with the manufacturer's recommendations. The scanner should routinely be assessed for quantitative integrity and stability by being tested using various imaging protocols on a standard phantom. For SUV measurements, this assessment should include a comparison against a dose calibrator to ensure accuracy; that is, a comparison of the absolute activity measured versus the measured activity injected, should be performed.

A daily QC check must be performed at the beginning of the day, including PET/MRI scanner and dose calibrator, in accordance with the manufacturer recommendations. If any of the QC results are outside of the manufacturer's guidelines, the study must be rescheduled and the problem rectified before scanning any patients.

5.5.5 ^{18}F-FES PET/MRI Imaging Acquisition
Image acquisition and reconstruction protocols specific to this research study will be available in a separate "Imaging Manual". The protocol is briefly described here.

The patient will be escorted to the GE Discovery 750W 3T MR scanner containing the PET insert and an 8-Channel breast coil qualified for PET/MRI located in the basement level of WIMR Tower 1. Directed breast PET/MRI data will be acquired with the patient in the prone position. Imaging will be performed by research technologists under guidance of the principal and/or collaborating investigators. MRI-sequences utilized in a standard clinical breast MRI will be obtained, including T2-weighted fat suppressed and diffusion weighted imaging (DWI) prior to gadolinium contrast administration followed by T1-weighted fat suppressed DCE imaging. The MRI sequences will include standard clinical sequences and may include research MRI sequences under development and evaluation at the University of Wisconsin. A standard MR-based attenuation correction algorithm provided by GE will also be used for this study. Breast specific PET emission data will be acquired simultaneous with the MR images. The entire examination is expected to take 30 – 40 minutes. Post-processing includes PET image reconstruction, correction for attenuation, scatter, and motion, and co-registration with the MR images.

In the rare instance that the subject is unable to be imaged using the simultaneous PET/MRI scanner (e.g. excessive girth, technical issue with scanner performance), the subject will undergo two separate scans. A breast MRI (approximately 30 to 40 minutes) will be performed using a 3T

breast MRI scanner (e.g. GE Signa Premier) using MRI sequences as described above, and a breast specific PET/CT scan will be performed using a PET/CT scanner (e.g. Discovery IQ or GE Discovery 710) located in the Department of Radiology in the Wisconsin Institutes for Medical Research (WIMR) Tower 1. The subject will be positioned prone with arms overhead. Following a CT scout (topogram), a directed low-dose, non-contrast, non-breathhold, non-diagnostic CT scan through the chest/breasts will be performed for attenuation correction calculations. Next, a static PET emission scan of the thoracic region to specifically cover the breasts will be acquired in 3D acquisition mode. The entire examination is expected to take approximately 15-20 minutes. Post-processing includes PET image reconstruction, correction for attenuation and co-registration with the CT images and also with the separately obtained breast MRI.

5.5.6 ^{18}F-FES PET/MRI Image Analysis

Images will be reviewed for general quality. The known biopsy-proven breast cancer(s) will be identified on the MRI/PET examination based on its known location from the prior clinical breast MRI, if performed, or the patient's previous imaging, as is typically done in routine clinical practice. MRI images will be used to localize the lesion(s), measure the lesion size (in three dimensions), and assign the region of interest (ROI) for PET imaging analysis.

Quantitative analysis of ^{18}F-FES uptake will be performed at the lesion-level. Standardized uptake values (SUV) will be measured by manually drawing a region of interest (ROI) to encompass the entire lesion guided by the lesion extent visualized on MRI. For the primary objective, the maximum SUV value (SUV_{max}) will be determined. For potential exploratory analyses, additional uptake values may include SUV_{peak} (small fixed-size ROI centered on most intense part of tumor), SUV_{mean} (average value), SUV_{min} (minimum value), SUV_{sd} (standard deviation), tumor-to-normal tissue uptake ratios, FTV (functional tumor volume; i.e. the volume of tumor tissue with increased ^{18}F-FES uptake) and TLU (total lesion uptake; i.e. the product of FTV × SUV_{mean}). A research workstation/software (e.g. Mirada Medical) will be used to perform these advanced quantitative techniques.

5.6 Clinical Laboratory Testing

A blood sample is to be obtained at the time of imaging (drawn prior to ^{18}F-FES injection) and submitted for hormonal analysis (estradiol and sex hormone binding globulin) by the UW Health clinical laboratory. Results from the UW Health clinical laboratory automatically post to the subjects' medical records.

5.7 Risk Assessment

Clinicopathologic-based risk scores will be calculated using the University of Southern California/Van Nuys Prognostic Index (21) and the Memorial Sloan-Kettering Cancer Center Nomogram (22).

Genomic-based risk scores will be calculated using the Oncotype DX DCIS test (25, 26). The risk score (0-100) is generated from expression levels of seven cancer related genes and five reference genes (**Table 3**) determined from a patient's biopsy or surgical specimen (25). Scores are further categorized as low-risk (0-38), intermediate-risk (39-54), and high-risk (55-100). Since utilization of the Oncotype DX DCIS score for clinical care is variable (only 1-5% of DCIS patients locally), we will perform an equivalent "research-based" assay using previously published methods (45). Tissue blocks from the surgical specimen will be identified and used for RNA isolation performed by the UW Translational Research Initiatives in Pathology (TRIP) Lab. The Fowler lab will then perform quantitative polymerase chain reaction (qPCR) published by Cronin et al (46) and will follow the algorithm of Solin et al (25) to calculate the genomic-based risk score. Results of this research assay will not be used to guide clinical management. A batched analysis will be performed after all subjects are enrolled for those with sufficient remaining tissue.

Table 3: 12 gene panel included in the Oncotype DX DCIS Score

Proliferation Group	Hormone Receptor Group	Other	Reference Group
Ki67	PR	GSTM1	ACTB (β-actin)
STK15			GAPDH
Survivin			RPLP0
CCNB1 (cyclin B1)			GUS
MYBL2			TFRC

5.8 Repeatability ^{18}F-FES PET/MRI (Optional)

Up to 5 patients with ER+ DCIS who agree to participate in this optional portion of the study will undergo a second (test-retest) ^{18}F-FES PET/MRI examination. The test-retest exam cannot be performed the same day but should be completed prior to any therapy or surgical excision. This will not delay any scheduled routine standard of care therapy or procedures for the patient. This optional sub-study will be offered to all patients at the time of consent until we have accrued up to 5 patients with ER+ DCIS.

6. REGISTRATION PROCEDURES

Patients must not start protocol procedures prior to registration. Patients must complete the breast PET/MRI study prior to surgical/oncologic intervention for their biopsy-proven breast cancer as part of their routine clinical care.

6.1 Patient Recruitment

Potentially eligible patients can be identified by several approaches: study coordinator(s) who have access to the clinical breast MRI schedule and the schedules from other providers that serve this patient population (e.g. surgeons), radiologists who are protocoling clinical breast MRI exams in advance, from patients presented at our weekly multidisciplinary and radiologic-pathologic

concordance conferences, and/or through automatic search results of the electronic medical record provided by the Biomedical Informatics Services delivered to the study investigator(s) and study coordinator(s) via REDCap database. The breast imaging radiologist performing radiologic-pathologic concordance after biopsy can also flag patients as potentially eligible.

Recruitment flyers will also be posted in clinics that serve this patient population. Patients interested in learning more about the research will be required to respond to a research team member(s) noted on the flyer.

Study eligibility will be based on the inclusion and exclusion criteria and will be determined by the breast imaging radiologist(s) and/or study coordinator(s). If a patient is potentially eligible for the study, the study coordinator will contact the patient by phone prior to their scheduled MRI and outline the research protocol to the patient. If the patient is interested in the study, the study coordinator will describe the study in more detail and/or record the subject's preferred contact information and make plans to follow-up with the patient by phone to describe the research protocol in more detail. If they choose, an encrypted version of the consent form will be emailed to them to allow additional time to review the form. If the patient indicates they are interested in proceeding with the study, scheduling for ^{18}F-FES production with the Radiopharmaceutical Production Facility will be coordinated with the patient's clinical breast MRI appointment.

If the patient is uncertain about participating in the study, they can be offered the contact information of an advocate member of the UWCCC Breast Cancer Research Advisory Network (BCRAN) to use as a resource for general information about participating in clinical research.

The consent process will occur prior to the administration of research procedures. During the COVID-19 pandemic, consent procedures will be conducted by phone, when possible, to minimize face-to-face contact subjects have with the research team. A copy of the consent form will be mailed or emailed to subjects prior to the scheduled consent phone call. If emailed, the consent form will be encrypted. A study team member will call potential subjects at the scheduled time to review the information in the consent form including study procedures, risks associated with participation, alternatives to participation and whom to contact for additional information. Any questions will be addressed during the course of the phone call and subjects will be encouraged to contact the study team with any questions or concerns they might have at any time. Upon completion of the consent process, a copy of the signed consent form will be provided in one of the following ways depending on each subject preferences and capabilities:

- Electronic signature will be provided using DocuSign
- Subject will be asked to scan a copy of the entire consent document and email it back to the study team.
- Subject will be asked to take a photo of the signature page and email a copy to the study team.

- If subjects are unable to provide an electronic copy of the signed consent form, they will be asked to bring a copy of the form to the research visit.

Once institutional requirements are met, remote consenting may continue by obtaining electronic signature using the DocuSign software platform.

6.2 Patient Enrollment

Study enrollment will occur when the patient arrives for and consents to the research study. A study team member will meet with the patient, review the research protocol, and answer the potential subject's questions. The information in the consent form, including study procedures, risks associated with participation, alternatives to participation and whom to contact for additional information, will be reviewed. Any questions will be addressed prior to the start of any research procedures and all subjects will be reminded that participation is optional and they can change their mind at any time and still get clinically required testing completed. Those interested in participating will be asked to sign informed consent and HIPAA authorization and the subject will be enrolled in the research study. Subjects will be assigned a unique ID number. Both the MRI screening questionnaire as well as the PET screening questionnaire will be reviewed to confirm eligibility criteria.

6.3 Eligibility Verification

The patient must meet all of the eligibility requirements listed in Section 4.0.

6.4 Registration Content Requirements

6.4.1 Protocol Number

6.4.2 Investigator Identification

- Investigator's name

6.4.3 Patient Identification

- Patient's name
- Patient's address
- Patient's Medical Record Number (MRN)
- Patient demographics
- Gender
- Birth date
- Race
- Ethnicity
- Date on study

6.5 Criteria for Removal from Study

Enrolled patients will be removed from the study in the following circumstances:

- Subject does not complete the PET/MRI examination per study protocol
- Positive pre-scan pregnancy test
- Subject withdraws consent
- Exclusion criteria are discovered after registration but prior to the PET/MRI examination

7. TREATMENT/INTERVENTION PLAN

This is an observational study and the results of the ^{18}F-FES PET/MRI will not be used to change patient therapy. The results of the research studies will not be reported by a radiologist, will not be part of the patient's medical record, and will not be available to the patient or their physician. The exception is if the breast MRI component of the breast PET/MRI is used as the patient's clinical examination, then interpretation of the breast MRI and management recommendations will be made based on routine clinical practice. No medications will be administered as part of the study protocol except intravenous injection of a gadolinium-based contrast agent for MRI and ^{18}F-FES PET tracer required for PET imaging.

7.1 Administration Schedule

For each research breast ^{18}F-FES PET/MRI examination, patients will receive:

- ^{18}F-FES, 6 mCi (222 MBq) ± 20%, IV slow infusion over 2 minutes followed by saline flush, once
- Gadolinum intravenous contrast (gadobenate dimeglumine; MultiHance), weight-based dosing (0.1 mmol/kg), IV power-injection at 2 cc/second followed by a 20 cc saline flush, once

7.2 Dose Modifications

There will be no dose modifications for ^{18}F-FES administration.

7.3 Supporting Care Guidelines

All supportive measures consistent with optimal patient care will be given throughout the study. Any adverse effects, related or non-related to the injection of ^{18}F-FES, will be treated as clinically indicated with no study-related restrictions.

If there is a contrast reaction or extravasation, these will be managed via UW Health guidelines. A physician is always present to manage contrast reactions and extravasations, should one occur.

To facilitate clearance of ^{18}F-FES, thus reducing patient radiation dose exposure, patients will be instructed to adequately hydrate prior to and after the radiopharmaceutical administration unless on a fluid restrictive diet by their treating physician.

7.4 Duration of Follow-up

Adverse events occurring within 24 hours after ^{18}F-FES infusion will be recorded by the study coordinator who will contact the subject within 1 to 3 days after ^{18}F-FES administration. Standard of care patient management will occur after the ^{18}F-FES breast PET/MRI examination. These visits generally include consultation with a surgeon, radiation oncologist, and/or medical oncologist. Subject's medical records are followed until completion of definitive treatment.

8. ADVERSE EVENT REPORTING REQUIREMENTS

8.1 Purpose

Adverse event (AE) data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of the patients enrolled, as well as those who will enroll in future studies using similar agents.

8.2 Terminology

- **Adverse Event (AE):** Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be **ANY** unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.
- **Attribution:** An assessment of the relationship between the adverse event and the protocol treatment, using the following categories.

ATTRIBUTION	DESCRIPTION
Unrelated	The AE is *clearly NOT related* to treatment.
Unlikely	The AE is *doubtfully related* to treatment.
Possible	The AE *may be related* to treatment.
Probable	The AE is *likely related* to treatment.
Definite	The AE is *clearly related* to treatment.

- **CAEPR (Comprehensive Adverse Events and Potential Risks List):** An NCI generated list of reported and/or potential AEs associated with an agent currently under an NCI IND. Information contained in the CAEPR is compiled from the Investigator's Brochure, the Package Insert, as well as company safety reports.
- **CTCAE:** The NCI Common Terminology Criteria for Adverse Events provides a descriptive terminology that is to be utilized for AE reporting. A grade (severity) is provided for each AE term.
- **Hospitalization (or prolongation of hospitalization):** For AE reporting purposes, a hospitalization is defined as an inpatient hospital stay equal to or greater than 24 hours.
- **Life Threatening Adverse Event:** Any AE that places the subject at immediate risk of death from the AE as it occurred.
- **Serious Adverse Event (SAE):** Any adverse event occurring at any dose that results in **ANY** of the following outcomes:
  - Death
  - A life-threatening adverse event

- Inpatient hospitalization or prolongation of existing hospitalization (for ≥ 24 hours).
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered a serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.3 Reporting Procedure

Per 21CFR312.32(c), all adverse events deemed both serious and unexpected associated with the use of the drug must be reported to the FDA and to all participating investigators as soon as possible and in no event later than 15 days.

The FDA will be notified of any unexpected fatal or life threatening experience associated with the drug as soon as possible but in no event later than 7 calendar days. The University of Wisconsin Health Sciences-IRB will be notified in accordance with posted institutional policy.

Serious and Non-Serious Adverse events will be recorded, regardless of whether or not they are thought to be related to the investigational imaging tracer. While all adverse events will be tabulated and reported in the study final report, serious adverse events will be reported in the course of the trial. Adverse events that meet criteria of a serious adverse event listed above will also be recorded and reported.

The Investigator will inform the IRB of all adverse events attributed to the investigational imaging tracer in accordance with posted institutional policy.

8.4 Additional Adverse Event Information

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

The following adverse event information was obtained from the NCI.

Condensed risk list – ^{18}F-FES PET

POSSIBLE, SOME MAY BE SERIOUS
• Swelling and redness at the site of the medication injection • Changes in taste

The only adverse events that have been attributed to PET imaging with diagnostic ^{18}F-FES at the levels described in the Investigators Brochure have been mild transient disturbances in taste. Therefore, no significant adverse events are expected as a result of the intravenous (IV) administration of ^{18}F-FES for typical PET imaging applications.

As with any IV administered agent, ^{18}F-FES could cause an allergic reaction that could potentially pose a threat to life (anaphylaxis). This has not been observed in reported human exposure to date. Reasonable precautions should be taken, consistent with normal radiologic and clinical facility practice. The patient should be monitored until the PET procedure is completed, and trained personnel and emergency equipment should be available per facility standards.

For purposes of informed consent regarding reasonably foreseeable risks to subjects in trials utilizing ^{18}F-FES, the following potential adverse events are considered extremely rare:

- Injection-related risks that may include infection, or accidental extravasation of the dose that may lead to discomfort, localized pain, or infection.
- Risks related to allergic reaction/anaphylaxis that may be life threatening.

As with all PET imaging agents, ^{18}F-FES is a radiopharmaceutical that decays with positron emission. As such, it poses an intrinsic radiation exposure risk. However, when administered in accordance with the Investigator's Brochure as a PET imaging agent, this risk is felt to be extremely small. The organ and total body doses associated with ^{18}F-FES PET imaging are comparable to or lower than those associated with other widely used clinical nuclear medicine procedures.

^{18}F-FES in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

9. MEASUREMENT OF TREATMENT OR INTERVENTION EFFECT

Not applicable since the research imaging study (PET/MRI) will not be used to guide therapy.

10. STUDY PARAMETERS

Information regarding time intervals for performance of the study and contact points is included as **Table 2** in Section 5.3.

11. DRUG FORMULATION AND PROCUREMENT

11.1 Gadolinium-based intravenous contrast agent

Gadobenate dimuglumine (MultiHance) is the gadolinium-based intravenous contrast agent used for the MRI portion of this study. It will be obtained from a commercial source (e.g. Bracco Diagnostics, Inc). This agent is used routinely in standard clinical practice at our institution. Please refer to package insert for more comprehensive information.

11.2 16α-[^{18}F]-fluoro-17β-estradiol (^{18}F-FES)

^{18}F-FES is the radiopharmaceutical which will be used for this study. For complete information, please refer to the Investigator's Brochure: "[^{18}F]Fluoroestradiol: An investigational positron emission tomography (PET) radiopharmaceutical for injection, intended for use as an in vivo diagnostic for imaging estrogen receptors in tumors (NCI-held IND 79,005)", Edition Number 4, Edition date 2011.

11.2.1 Other Names

[F-18]FES, FES

11.2.2 Classification

Investigational new drug: Radiopharmaceutical/radiotracer

In May 2020, ^{18}F-FES was approved by the FDA under the brand name "CERIANNA", but the labelled indication for clinical use is for a different breast cancer patient population (recurrent or metastatic) than included in this research study (DCIS). Thus, we will continue to perform this research study under our existing approved IND.

11.2.3 Mode of Action

^{18}F-FES binds to ER alpha with a binding affinity nearly identical to estradiol. ^{18}F-FES binding to sex steroid binding protein (SBP or SHBG) is also similar to that of estradiol, providing equivalent transport to sites of ER expression, including tumor sites. In breast cancer, the uptake of ^{18}F-FES, measured by whole body PET/CT, has been shown to correlate with ER expression in biopsy material assayed by *in vitro* radioligand binding or by IHC.

11.2.4 Storage and Stability

In accordance with regulations, the Radiopharmaceutical Production Facility conducts several quality control tests on the ^{18}F-FES product prior to release for human administration. Once delivered, doses will be stored in the appropriate storage area in the nuclear medicine facility until they are administered to the patient. The drug solution is stored at room temperature in a gray butyl septum sealed, sterile, pyrogen-free glass vial with an expiration time of 6 hours.

11.2.5 Dose Specifics

The injected dose of ^{18}F-FES will be 6 mCi (222 MBq) ± 20% with a specific activity greater than 170 Ci/mmol at the time of injection for an activity dose of 6 mCi. In all cases, less than 5 µg of ^{18}F-FES should be injected. ^{18}F-FES is the only active ingredient. There is no evidence that nonradioactive and radioactive FES molecules display different biochemical behavior.

11.2.6 Preparation

Fluorine-18 labeled FES is synthesized with high specific activity so the quantity of estrogenic material injected with the radiopharmaceutical is ≤ 5 µg (≤ 17 nmol). For a 6 mCi dose, this would be 170 Ci/mmol. Between 3 and 6 mCi of [^{18}F]FES is administered in a nominal volume of 10 mL of phosphate buffered saline containing less than 15% ethanol (v:v) for a single PET scan.

Specific manufacturing processes will follow our approved Drug Master File for ^{18}F-FES. Briefly, [^{18}F]FES is purified by HPLC using an eluent of 50% ethanol, USP in sterile water for injection. The final product [^{18}F]FES with a total volume of 10 mL is formulated in 10% ethanol for injection, USP and 0.075M saline for injection, USP, buffered with 0.075M sodium phosphates injection, USP. The concentration of ethanol in the final injectate is less than 15% by volume, or a maximum of 1.5 mL of ethanol. This is less than one-sixth of the amount of ethanol in one alcoholic drink and < 0.025 mL/kg (< 0.02g/kg) for a standard 56.8-kg woman.

11.2.7 Route of Administration

^{18}F-FES is a sterile, intravenously (IV) injectable solution in a volume of ≤ 10 mL containing of 0.15 M phosphate buffered saline: < 15% ethanol (v:v). Prior studies have shown that this volume of injectate infused over 2 minutes is well tolerated and minimizes local saturation of SHBG which could limit systemic delivery of ^{18}F-FES.

11.2.8 Incompatibilities

N/A

11.2.9 Availability

cGMP-grade ^{18}F-FES will be manufactured on-site in the UW-Madison Radiopharmaceutical Production Facility. This facility has been qualified and approved for production by NCI Cancer Imaging Program (CIP) and ECOG-ACRIN for other FES clinical trials. The investigational pharmacist or qualified nuclear medicine technologist will be the responsible party designated by the investigator.

11.2.10 Side Effects

See Section 8.4 for side effects. The radiation absorbed effective dose equivalent to the whole body from intravenously injected ^{18}F-FES is estimated to be 0.022 mSv/MBq (488 mrem for a 6 mCi injection). The critical organ is the liver, with an average absorbed dose of 0.13 mGy/MBq. The organ and total body doses associated with ^{18}F-FES PET imaging are comparable to or lower than those associated with other widely used clinical nuclear medicine procedures and are well below the maximum suggested individual study and annual total body dose of 30 and 50 mGy, respectively, suggested for investigational radiopharmaceuticals by the FDA.

11.2.11 Nursing/Patient Implications

Standard safety precautions required when handling radioactive materials (predominantly during the injection and uptake period) should be followed. ^{18}F-FES requirements are similar to those used for other PET tracers, such as ^{18}F-FDG.

11.3 Cerianna (fluoroestradiol F-18)

If scheduling conflicts do not permit use of locally produced ^{18}F-FES, then commercially available ^{18}F-FES (CERIANNA) will be obtained which is FDA approved for patients with recurrent or metastatic ER+ breast cancer and is being used in a manner that is consistent with its approval.

The use of this product in research is consistent with the criteria for IND exemption in accordance with 21 CFR 312.2(b)(1). Cerianna is a lawfully marketed drug, and this research is not intended to support a new indication or new product labeling.

12. STATISTICAL CONSIDERATIONS

12.1 Primary Objective

To compare quantitative ^{18}F-FES uptake of biopsy-proven DCIS measured using PET/MRI with ER protein levels determined by immunohistochemistry.

The primary objective is to compare ^{18}F-FES SUV_{max} from PET/MRI with the ER immunohistochemistry semi-quantitative score obtained by using the Allred score. We will test the hypothesis that ^{18}F-FES SUV_{max} from PET/MRI correlates well against the ER IHC Allred score. The correlation of the two measures will be evaluated using Spearman correlation. The null hypothesis is H_0: p_0=0.00 and the alternative hypothesis is H_1: p_1 =0.73.

A sample size of 12 patients achieves 82% power (β=0.20) for detecting an expected correlation coefficient (r=0.73) to be significantly different from zero with two-tailed α value of 0.05 (http://www.sample-size.net/correlation-sample-size/). We anticipate enrolling 1 subject every 1-2 months. The number of subjects with ER-negative DCIS will be limited to ≤3.

Other measures of ^{18}F-FES uptake including SUV_{peak}, SUV_{mean}, tumor-to-normal tissue uptake ratio, and total lesion uptake will also be correlated with the ER immunohistochemistry semi-quantitative score obtained by using the Allred score.

12.2 Secondary Objectives

1. To determine the optimal cut-point ^{18}F-FES uptake value for distinguishing between ER+ and ER-negative DCIS.

   Receiver operating characteristic (ROC) curve analysis will be performed to determine the optimal cut-point for ^{18}F-FES uptake to distinguish ER+ from ER-negative DCIS, as defined by the clinical pathology report. The area under the curve (AUC) for the ROCs and their respective two-sided 95% confidence intervals will be calculated using logistic regression. The optimal cut-off point will be determined by considering the ^{18}F-FES uptake value with the maximum sensitivity and specificity.

2. To determine the test-retest reproducibility of quantitative assessment of tumor ^{18}F-FES uptake.

   The reproducibility of the two measurements (test-retest) of ^{18}F-FES uptake in the subjects (up to 5) who elect to undergo a second imaging session will be assessed by intra-class correlation coefficient (ICC) and its 95% confidence interval (47). Additionally, the coefficient of repeatability (CR) and the Bland-Altman plot will be used (48).

3. To determine the optimal cut-point ^{18}F-FES uptake value for distinguishing between low-risk DCIS and intermediate/high-risk DCIS.

ROC curve analysis will be performed to determine the optimal cut-point for ^{18}F-FES SUV_{max} to distinguish low-risk DCIS and intermediate/high-risk DCIS. Risk categories will be determined using the Van Nuys Prognostic Index, the Memorial Sloan-Kettering Cancer Center Nomogram, and the research-based Oncotype DX DCIS score. Sensitivity and specificity will be determined with two-sided 95% confidence intervals. The AUCs for the ROCs and their respective two-sided 95% confidence intervals will be calculated using logistic regression.
The optimal cut-off point will be determined by considering the ^{18}F-FES uptake value with the maximum sensitivity and specificity.
This analysis will be done separately for each risk assessment model.

4. To estimate the association of quantitative ^{18}F-FES uptake (continuous SUV_{max}) with research-based Oncotype DX DCIS scores (0-100).

   Scatter plots of continuous quantitative ^{18}F-FES uptake (SUV_{max}) on the y-axis and research-based Oncotype DX DCIS scores (unitless) on the x-axis will be created to explore the distribution of the measurements. Pearson's or Spearman's rank correlation will be used to evaluate the association between quantitative ^{18}F-FES uptake and research-based Oncotype DX DCIS score. The correlation coefficient (rho) and 95% confidence interval will be reported.

5. To determine the upgrade rate to invasive cancer at surgical excision.

   This percentage will be calculated by dividing the number of patients with invasive breast cancer diagnosed at the time of surgical excision by the number of patients with percutaneous biopsy-proven DCIS in the study.

6. To correlate tumor ^{18}F-FES uptake with serum estradiol and sex hormone binding globulin levels.

   A correlation analysis of serum estradiol and sex hormone binding globulin levels will be performed using Pearson's or Spearman's rank correlation. Scatter plots, correlation coefficients (rho) and 95% confidence intervals will be reported.

12.3 Secondary Objectives

To correlate tumor cell density with ^{18}F-FES uptake on PET/MRI. A strong correlation between tumor cell density and detection by ^{18}F-FDG PET/CT

imaging has been reported (49). A correlation analysis of tumor ^{18}F-FES uptake and tumor cell density will be performed using Pearson's or Spearman's rank correlation. Scatter plots, correlation coefficients (rho) and 95% confidence intervals will be reported.

12.4 Evaluation of Toxicity

All subjects will be evaluable for toxicity from the time of injection of ^{18}F-FES until 24 hours after injection. For this evaluation, the study coordinator will contact the subject within 1 to 3 days after ^{18}F-FES administration.

13. PATHOLOGY REVIEW

13.1 Justification

The pathology of the biopsy-proven breast cancer will be evaluated per the current standard of care (histologic diagnosis, grade, tumor biomarker status). ER status is reported as positive or negative and includes a percentage of cells staining positive as well as intensity of staining. This information will be obtained from the clinical pathology report in the subjects' electronic medical record and will be used to calculate a semi-quantitative Allred score (44). If the information needed to calculate the Allred score is not included in the clinical pathology report, a pathologist on the research study team will review the existing ER IHC slides. If the information needed to determine tumor cell density is not included in the clinical pathology report, a pathologist on the research study team will review the existing H&E slides.

13.2 Required Pathology Materials

Pathology reports will be available through the electronic medical record. Existing pathology slides (ER IHC and H&E) from the breast biopsy used for initial diagnosis will be requested from the Department of Pathology for Allred scoring by the research study pathologist if the information needed to calculate the Allred score is not included in the clinical pathology report.

For the research-based Oncotype DX DCIS score, a study pathologist will review the H&E slides from the surgical specimen and select a block for release to the TRIP Laboratory. We anticipate needing 6 unstained sections per patient specimen for sufficient RNA isolation for the assay.

13.3 Routing

If needed, requested pathology slides will be sent to the research study pathologist.

14. RECORDS TO BE KEPT

The PI will supervise the data collection and management. The following types of data and materials will be collected:

14.1 Images

Images from the PET/MRI examinations will be stored on the local picture archiving and communication system (PACS) (McKesson, San Francisco, CA). The local PACS system is password protected behind the UWHC Department of Radiology firewall.

14.2 Regulatory and Consent

A research chart will be created for each subject. The chart will include the signed consent form, a copy of the subject's experimental bill of rights, and a signed HIPAA form. Research charts will be kept in a locked file cabinet within a study team members secure office. An enrollment log will be maintained. The enrollment log will confirm that informed consent was obtained on each subject. The patient has the right to decline to participate in the study and to request removal from the study at any time. Informed consent will be provided and signed prior to enrollment in the study. Patients may refuse to answer any questions asked during the duration of the study if they are too uncomfortable to answer. All of these potential risks will be described to the patient in the informed consent document.

14.3 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Subjects will be assigned a unique study number upon enrollment that will be used to label their research data in lieu of directly identifiable information. All subject data collected during participation in the study will be kept in a locked file cabinet within a secure office or a password-protected database that uses departmental servers and will only be accessible to study team members. After the study is completed, all data will be de-identified for the purposes of research presentations/publications. The study breast MRI examinations will be archived on the standard clinical PACS system which is password protected behind the UWHC firewall. The study data and records will be maintained for up to 5 years after the conclusion of the study, at which point the PI will destroy the key to the coded identifiers, thus permanently anonymizing the data.

15. PATIENT CONSENT AND PEER JUDGMENT

Current FDA, state, federal, and institutional regulations concerning informed consent will be followed.

16. DATA AND SAFETY MONITORING

Safety monitoring of research protocols investigating novel PET tracers utilize a combination of tools. These are framed to provide adequate clinical trial oversight ensuring that the research team is complying with the conditions of IRB approval and FDA requirements. The tools enable frequent evaluations of study-related activities, identification of deviations or noncompliance with the conduct of the study or protocol, review of adverse events tracking and reporting, and review of drug production and accountability.

These tools include:

- Study visit checklists – Research Coordinators use study visit checklists, developed in conjunction with the PI, to ensure that all study required procedures and processes are met before, during and after the subject has been enrolled. Items listed on these checklists include informed consent obtained, consent questions answered (and by whom), medications reviewed, MR safety form, etc. These checklists are completed and reviewed by the study coordinator requiring signature approval for each study subject to ensure tight adherence to the protocol.

- Safety monitoring – The safety monitoring of subjects enrolled in the research includes both internal and external safety checks governing the administration of ^{18}F-fluoroestradiol (FES). Internal monitoring is performed by co-investigators and external monitoring by a team of four physicians, scientists, and staff with the appropriate broad clinical and technical expertise. The internal monitoring plan includes a brief review of each subject visit after it occurs. In this regard, the study team will discuss data quality, ^{18}F-FES production and administration, and subject compliance and tolerance of research procedures.

  The external monitoring plan includes a 4-person committee of physicians, scientists, and staff with the appropriate expertise (nuclear medicine, oncology, research nurse manager, medical physics) that meets annually with representatives from the research team, to discuss research progress, AE reports, as well as other data reports (note-to-file documentation, protocol deviations, reportable events) compiled by the study team. If an AE occurs, an ad hoc committee meeting will be organized to discuss whether the event is considered serious, whether it can be attributed to research procedures, whether it constitutes an unanticipated problem or non-compliance on the part of the study team, and the plan for resolution and a future remediation.

  As part of this plan, the monitor(s) will verify that:
  - The rights and well-being of human subjects are protected
  - Reported study data are accurate, complete, and verifiable from source documents
  - The conduct of the study is in compliance with the currently approved protocol, GCPs, applicable regulatory requirements, and guidelines for clinical research studies at the University of Wisconsin-Madison and its affiliates

16.1 Risks

16.1.1 MRI

The risk from the MRI component of the exam is minimal and is primarily related to claustrophobia and discomfort with positioning. Only staff skilled in the placement of IVs will place IVs for the study breast PET/MRI. Injection of gadolinium contrast has common mild side effects, including nausea, headache, and hives. More serious reactions, while rare, can occur, including allergic reactions. Any significant allergic reaction or contrast extravasation will be monitored and treated appropriately by clinical staff.

All hardware used to obtain MR images is FDA approved and will be used in accordance with the conditions approved by FDA. The investigational software being used in image acquisition is designed to stay within the current guidelines for MRI safety, established by the FDA. In addition, the investigational software does not meet the definition of a Significant Risk Device as outlined by the FDA under 21 CFR 812.3 as being:

- Intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject;
- Purported or represented to be for use supporting or sustaining human life and presents a potential for serious risk to the health, safety or welfare of a subject;
- For a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety or welfare of a subject; or
- Otherwise presents a potential for serious risk to the health, safety, or welfare of a subject.

16.1.2 PET

^{18}F-FES has been safely administered to more than 1,000 healthy volunteers and breast cancer patients participating in clinical trials worldwide as of 2013. In May 2020, 18F-FES was approved by the FDA under the brand name “CERIANNA”, but the labelled indication for clinical use is for a different breast cancer patient population (recurrent or metastatic) than included in this research study (DCIS). Thus, we will continue to perform this research study under our existing approved IND. The only adverse events that have been attributed to ^{18}F-FES at the levels described in the Investigators Brochure have been mild transient disturbances in taste and injection site pain.

Risks associated with PET imaging result primarily from the radiation dose from the radiopharmaceutical. For this research study, approximately 6 mCi of ^{18}F-FES will be administered intravenously which is also the standard dose used for other clinical trials using ^{18}F-FES. The radiation absorbed effective dose equivalent to the whole body from intravenously injected FES

is estimated to be 0.022 mSv/MBq (4.88 mSv for a 6 mCi injection). The critical organ is the liver, with an average absorbed dose of 0.13 mGy/MBq. For context, the average person in the United States receives an estimated effective dose of about 3 mSv per year from naturally occurring radioactive materials, such as radon and radiation from outer space. The organ and total body doses associated with FES PET imaging are comparable to or lower than those associated with other widely used clinical nuclear medicine procedures and are well below the maximum suggested individual study and annual total body dose of 30 and 50 mGy, respectively, suggested for investigational radiopharmaceuticals by the FDA (50).

16.1.3 Unexpected findings

All patients in this study will receive the clinical "gold standard", diagnostic breast MRI and ER measurement via IHC, as part of their clinical care as a requirement for enrollment in the study. Thus, the breast specific ^{18}F-FES PET data is not expected to yield any unexpected findings. Subjects will not be informed of any unexpected findings on the ^{18}F-FES PET/MRI imaging.

16.1.4 CT (performed only in rare instance when subject is unable to be imaged using the simultaneous PET/MRI scanner)

Risks associated with CT include exposure to 1.5 mSv of radiation from the low-dose chest CT scan required for attenuation correction calculations. Thus, the total whole body effective dose equivalent for breast PET/CT is approximately 6.5 mSv. This corresponds to approximately 2 years of natural background radiation and remains well below the annual occupational dose limit for radiation workers.

Additionally, subjects may experience discomfort while lying in the PET/CT scanner.

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