[^{18}F]Fluorofuranylnorprogesterone (FFNP) PET/MR Imaging of Progesterone Receptor Expression in Invasive Breast Cancer

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ABBREVIATIONS

CT	Computed tomography
DCE-MRI	Dynamic contrast enhanced magnetic resonance imaging
ER	Estrogen receptor
FDA	U.S. Food and Drug Administration
FDG	^{18}F-fluorodeoxyglucose
FFNP	^{18}F-fluorofuranylnorprogesterone
HER2	Human epidermal growth factor receptor 2
IHC	Immunohistochemistry
IRB	Institutional review board
mCi	MilliCurie
MIRS	Medical Imaging Research Support
MRI	Magnetic resonance imaging
PACS	Picture archiving and communication system
PET	Positron emission tomography
PR	Progesterone receptor
ROC	Receiver operating characteristic
ROI	Region of interest
SUV	Standard uptake value
UW	University of Wisconsin

1. PROTOCOL SUMMARY

TITLE OF STUDY
[^{18}F]Fluorofuranylnorprogesterone (FFNP) PET/MR Imaging of Progesterone Receptor Expression in Invasive Breast Cancer

CLINICAL PHASE
Phase 2

INVESTIGATORS
Lead clinical investigator: Amy Fowler, MD, PhD, University of Wisconsin-Madison
Clinical co-investigators: Roberta Strigel, MD, MS; Kari Wisinski, MD; Lee Wilke, MD, Aparna Mahajan, MD; Stephanie McGregor, MD, PhD, University of Wisconsin-Madison

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

PERIOD OF TRIAL
Planned study conduct duration of approximately 36 months.

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 test the accuracy of PET/MRI imaging with ^{18}F-fluorofuranylnorprogesterone (FFNP) for measuring progesterone receptor (PR) expression in patients with invasive breast cancer.

Primary Objective

To compare FFNP uptake of biopsy-proven primary PR+ breast malignancies measured using PET/MRI with the reference standard of PR immunohistochemistry (IHC).

Secondary Objectives

1) To determine the test-retest reproducibility of quantitative assessment of tumor FFNP uptake; 2) to determine the intra- and inter-observer variability of quantitative assessment of tumor FFNP; 3) to evaluate the optimal cut-point of FFNP uptake for distinguishing between PR-negative and PR-positive invasive breast cancer; and 4) estimate the association of tumor FFNP uptake (continuous SUV_{max}) with research-based Oncotype DX scores (0-100).

Exploratory Objectives

1) To evaluate heterogeneity of tumor FFNP uptake; 2) to correlate tumor FFNP uptake with serum progesterone and estradiol levels; and 3) to correlate tumor FFNP uptake with disease recurrence.

INCLUSION CRITERIA

- Women 18 years of age or older
- Diagnosis of biopsy-proven invasive breast cancer measuring at least 1.0 cm in diameter by any imaging modality
- Biopsy-proven PR-positive) or PR-negative invasive breast cancer
- 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
- Patients who have had neoadjuvant chemotherapy/endocrine therapy, surgical intervention, or radiation for the current biopsy-proven malignancy
- Patients with breast expanders
- Patients who are or might be pregnant or lactating
- Patient girth exceeds the bore of the PET/MRI scanner
- Patients with a contraindication to gadolinium based contrast agents, including allergy or impaired renal function (per UW Health Guidelines)
- Patients with a history of allergic reaction attributable to compounds of similar chemical or biologic composition to FFNP
- Patients in liver failure as judged by the patient's physician
- Patients with standard contraindications to MRI, including claustrophobia and metallic implants incompatible with MRI
- 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
- Patients unable to lie prone for 30 minutes for imaging

STUDY DESIGN

This is a prospective, one-arm, study which will enroll patients with newly diagnosed breast cancer 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 the investigational radiopharmaceutical, FFNP. FFNP uptake of the known, biopsy-proven malignancy will be measured using standardized uptake values (SUV) and tumor-to-normal tissue ratios. The proposed work-flow is described in the schema below.

NUMBER OF SUBJECTS

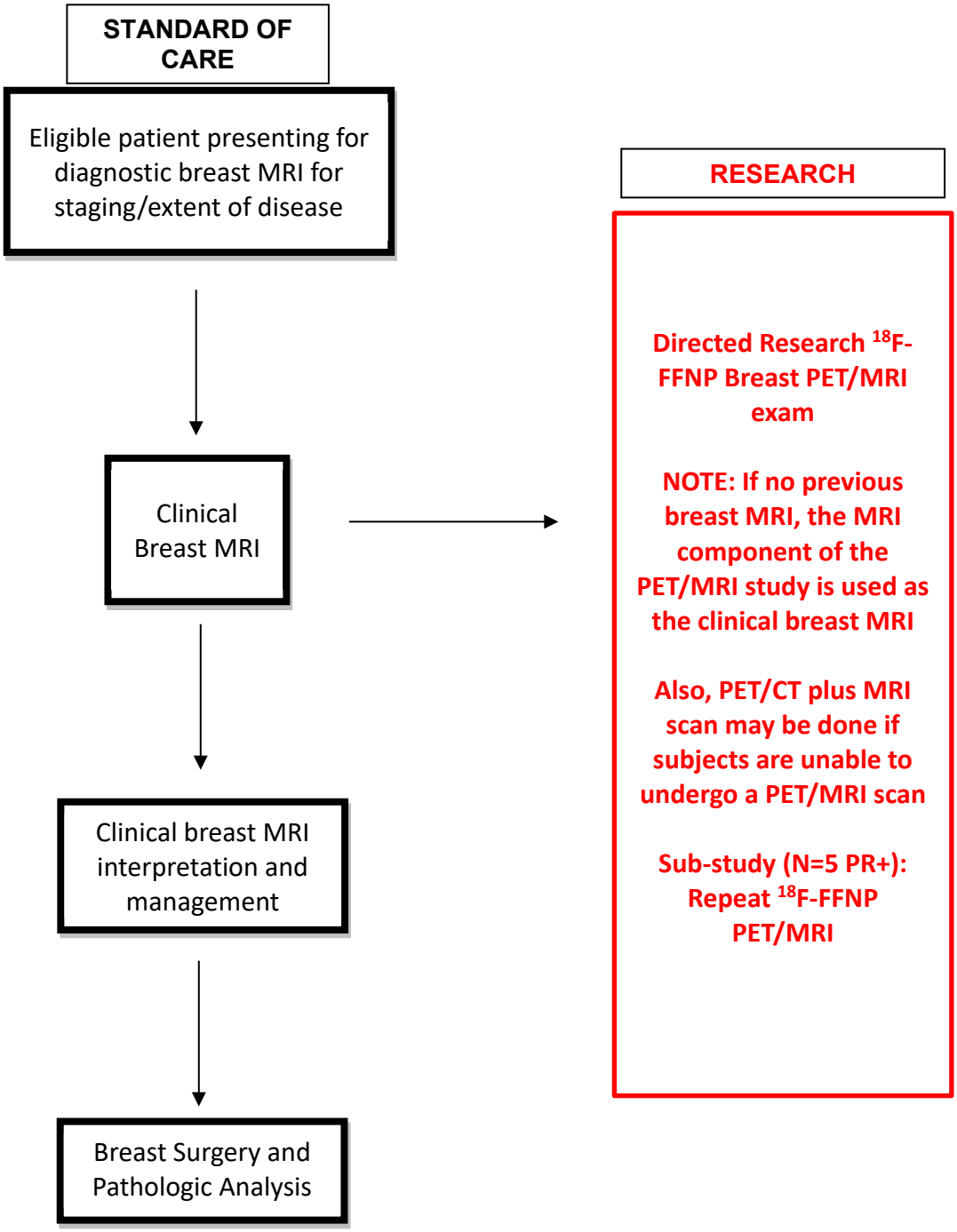
* Patients with newly diagnosed invasive breast cancer from a screening breast MRI are eligible to participate in this research. For these subjects, the research breast PET/MRI should be performed prior to any treatment or surgery for the invasive breast cancer.

The primary objective is to compare FFNP SUV_{max} from PET/MRI with the PR immunohistochemistry semi-quantitative score obtained by using the Allred score (0-8) (*1*). The correlation of the two measures will be evaluated with Pearson's correlation coefficient. The null hypothesis is H_0: p_0=0.20 and the alternative hypothesis is H_1: p_1 =0.70.

A sample size of 21 patients achieves 80% power to detect this difference, using a one-sided hypothesis test with a Type I error of 5%, accounting for 1 dropout. Up to 5 subjects with PR-negative breast cancer will be enrolled.

To measure test-retest reproducibility, a subset of up to 5 PR+ participants will have test-retest FFNP scans.

SCHEMA


STANDARD OF CARE
Eligible patient presenting for diagnostic breast MRI for staging/extent of disease
Clinical Breast MRI
Clinical breast MRI interpretation and management
Breast Surgery and Pathologic Analysis
RESEARCH
Directed Research 18F-FFNP 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 scan may be done if subjects are unable to undergo a PET/MRI scan
Sub-study (N=5 PR+): Repeat 18F-FFNP PET/MRI

2. INTRODUCTION

The majority of deaths due to breast cancer occur in women with estrogen receptor alpha positive (ER+) disease (*2*). Endocrine therapy targets the estrogen signaling pathway controlling tumor growth and is standard treatment for women with ER+ primary and metastatic breast cancer. However, endocrine therapy is effective in some but not all women with ER+ tumors. Due to the well-recognized diversity and complexity of ER signaling mechanisms, it is inevitable that some patients will have or develop disease that has circumvented standard methods for blocking ER function and have become endocrine insensitive. This important shift in receptor functionality cannot be directly measured by current clinical diagnostic approaches. Thus, a reliable test that measures functional endocrine sensitivity in a single patient is imperative and has potential for significant clinical impact.

Progesterone receptor (PR) is a classic estrogen-regulated target gene (*3*). Measuring changes in PR expression may be a useful pharmacodynamic biomarker for ER function and endocrine sensitivity. Howell et al. showed using tissue biopsy that changes in PR protein levels within a few weeks after initiating anti-estrogen therapy with tamoxifen in patients with locally advanced or recurrent ER+ breast cancer correlated with prolonged time to progression and improved survival (*4*). When analyzing the PR status using only the pretreatment biopsy, no significant differences in survival or time to progression were identified. Thus, PR can serve as a pharmacodynamic biomarker of endocrine sensitivity and ERα functional activity.

Tumoral expression of PR can be visualized and quantified through the use of an investigational radiolabeled progestin, ^{18}F-fluorofuranylnorprogesterone (FFNP), and positron emission tomography (PET) imaging (*5-10*). A “first-in-human” study involving 20 patients with invasive breast cancer has been published using FFNP-PET/CT imaging (*11*). There were no adverse events, changes in vital signs, laboratory values or electrocardiograms, or clinically detectable pharmacologic effects. Dosimetry evaluation determined that the effective dose equivalent of FFNP is comparable to other clinically utilized PET imaging agents. FFNP uptake as measured by tumor-to-normal breast tissue ratios was greater in PR+ cancers compared to PR- cancers. Thus, FFNP-PET appears to be a feasible method of imaging PR in breast cancer patients.

A drawback of PET for imaging primary breast cancer is its limited spatial resolution. Magnetic resonance imaging (MRI), however, produces exquisitely detailed images of the breast due to its high spatial resolution. MRI evaluation of breast cancer is dependent on anatomic detail to characterize tumor morphologic characteristics and functional assessment of lesion perfusion, characterized by the time-dependent uptake and lesion enhancement with gadolinium contrast (dynamic contrast enhanced MRI). These features make breast MRI the most sensitive method for the detection of breast cancer (*13*). In contrast, CT does not offer the soft tissue differentiation necessary for evaluation of the breast. Thus, MRI has become the standard of care when an additional modality is necessary to evaluate for breast cancer. CT is not used in clinical practice to evaluate the breasts.

Combining PET with MRI leverages the benefits of both modalities for the evaluation of breast cancer. Simultaneously acquired MRI images assists with PET radiotracer dose reduction, which is a necessary, critical advancement for the translation of PET/MRI imaging of the breast to clinical practice. Our research team has developed and validated the imaging acquisition protocol and workflow for breast PET/MRI (*14*). The protocol accounts for MR attenuation correction, quantitative standardized uptake value (SUV) calculation using PET, motion correction, co-registration of the two imaging modalities, radiopharmaceutical dose reduction, and patient workflow considerations. Although this protocol was validated using ^{18}F-fluorodeoxyglucose (FDG), it will be applicable for any novel molecular radiotracer to be imaged in combination with MRI, including FFNP.

It is important to validate the accuracy and reproducibility of quantitative tumor FFNP uptake measured using PET/MRI breast imaging. Further studies will be performed to test whether FFNP-PET/MRI can be used to distinguish patients with endocrine-sensitive from endocrine-resistant ER+ primary breast cancer. To the best of our knowledge, this study will be the first to investigate simultaneous breast PET/MRI imaging with FFNP for measuring PR expression in primary breast cancer.

3. STUDY AIMS/STUDY OBJECTIVES

The goal of this research is to test the accuracy of PET/MRI imaging with ^{18}F-fluorofuranylnorprogesterone (FFNP) for measuring progesterone receptor (PR) expression in patients with invasive breast cancer. We hypothesize that FFNP SUV_{max} from PET/MRI will correlate well against the semi-quantitative PR immunohistochemistry score.

3.1 Primary objective:

To compare FFNP uptake of biopsy-proven primary PR+ breast malignancies measured using PET/MRI with the reference standard of PR immunohistochemistry (IHC).

3.2 Secondary objectives:

- To determine the test-retest reproducibility of quantitative assessment of tumor FFNP uptake.
- To determine the intra- and inter-observer variability of quantitative assessment of tumor FFNP.
- To evaluate the optimal cut-point of FFNP uptake for distinguishing between PR-negative and PR-positive invasive breast cancer.
- Estimate the association of tumor FFNP uptake (continuous SUV_{max}) with research-based Oncotype DX scores (0-100)

3.3 Exploratory objectives:

- To evaluate heterogeneity of tumor FFNP uptake.
- To correlate tumor FFNP uptake with serum progesterone and estradiol levels.
- To correlate tumor FFNP uptake with disease recurrence.

Study duration: 36 months. Our estimated rate of accrual is 1 participant per month.

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 invasive breast cancer 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 invasive breast cancer measuring at least 1.0 cm in diameter by any imaging modality

4.1.3 Biopsy-proven PR-positive or PR-negative invasive breast cancer

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

Exclusion Criteria:

4.1.5 Inability or unwillingness to provide informed consent to the study

4.1.6 Patients who have had neoadjuvant chemotherapy/endocrine therapy, surgical intervention, or radiation for the current biopsy-proven malignancy

4.1.7 Patients with breast expanders

4.1.8 Patients who are or might be pregnant or lactating

4.1.9 Patients with a contraindication to gadolinium based contrast agents, including allergy or impaired renal function (per UW Health Guidelines)

4.1.10 Patients with a history of allergic reaction attributable to compounds of similar chemical or biologic composition to FFNP

4.1.11 Patients in liver failure as judged by the patient's physician

4.1.12 Patients with standard contraindications to MRI, including claustrophobia and metallic implants incompatible with MRI

4.1.13 Patients requiring 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:

4.1.13.1 The patient has their own prescription for the medication

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

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

4.1.13.3 The patient comes to the research visit with a driver.

4.1.14 Patients unable to lie prone for 30 minutes for imaging

5. RESEARCH DESIGN AND METHODS

5.1 Study Design

This prospective, one-arm, single-institutional research study will enroll patients with invasive breast cancer scheduled for diagnostic breast MRI for preoperative staging/extent of disease evaluation. Eligible patients will be consented for participation in the research study which will be performed on a separate day from the clinical breast MRI, if already performed, but prior to surgery. 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. The results of the FFNP-PET/MRI 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 score will be obtained from the surgical specimen for research purposes only. The proposed work-flow is described in the schema above.

5.2 Reference Standard

The primary objective is to measure FFNP uptake of biopsy-proven primary PR+ invasive breast cancer using PET/MRI. The reference standard will be PR expression measured with immunohistochemistry performed as per the current standard of care.

At UW Health, PR status is routinely reported as positive or negative and includes a visual estimation of the percentage of cells staining positive as well as intensity of staining. This information will be obtained from the pathology report in the subject's electronic medical record and will be used to calculate a semi-quantitative Allred score (*1*). 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 PR IHC slides. Comparison of FFNP uptake with PR IHC expression will be performed as previously described for ^{18}F-fluoroestradiol and ER IHC (*15*).

The Allred score is the sum of the proportion score and the intensity score (**Table 1**) (*1*). PR-negative cancers can have either an Allred score of 0 or 2 (1+1). PR-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		X
Radiology Reports[d]	X		X
Pathology Reports (Breast biopsy and final surgical excision)[d]	X		X
Concomitant Medication Review		X	
Pre-Scan Pregnancy Test (if needed)		X	
Day of last menstrual period (if premenopausal)		X	
Height and Weight		X	
Vital Signs[e]		X	
Blood for Hormone Levels		X	
FFNP-PET/MRI[i]		X[j]	
Adverse Event Evaluation[f]		X	X
Research-based Oncotype DX Score[g]			X

(a) Baseline is defined as the time period from initial subject contact until injection of FFNP.
(b) Imaging Day is defined as the day of the research FFNP-PET/MRI session.
(c) Any standard of care clinical visits, labs, or imaging exams obtained during this time period will be reviewed from the electronic medical record to correlate with scan results.
(d) Clinical data, including age, primary breast cancer data (standard clinical histopathology including ER/PR/HER2/Ki-67, location, size, and date of biopsy/diagnosis), Oncotype Dx recurrence score (if obtained clinically), mammographic breast density, and menopausal status will be recorded. If premenopausal, menstrual cycle data will be recorded.
(e) Vitals signs (blood pressure, heart and respiratory rate, and temperature) will be measured before FFNP injection, after FFNP injection, and after the PET/MRI scan.

(f) Adverse events occurring within a 24-hour period post-FFNP infusion will be recorded by the study coordinator who will contact the subject within 1 to 3 days.
(g) A research-based Oncotype DX assay will be performed using the resection specimen, if remaining tissue is available.
(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 scan in addition to separate MRI scan.
(j) For subjects participating in the test-retest cohort, the second scanning session will be done 24 hours apart or within a maximum 14-day time frame.

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 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 Imaging (FFNP- PET/MRI)

5.5.1 Timing of Research Breast Imaging Session

Patients must complete the research imaging session 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 PET/CT imaging with FDG for standard of care clinical staging evaluation, the research imaging session cannot be performed on the same day as FDG-PET/CT due to the half-life of ^{18}F.

If patients are also scheduled for lymphoscintigraphy using ^{99m}Tc-based radiopharmaceuticals, the research study should be scheduled on a separate day prior to the lymphoscintigraphy study.

5.5.2 Patient Preparation

The patient does <u>not</u> need to be fasting for FFNP-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 and vital signs (blood

pressure, heart and respiratory rate, and temperature) will be measured and recorded.

5.5.3 FFNP Administration

The patient will have an intravenous line placed, typically in the hand or arm opposite to the known primary breast cancer, FFNP will be given by a slow infusion (approximately 2 minutes), and the dose administered will be approximately 7 mCi. The injection site will be inspected by the technologist to assess for any possible infiltration. Vital signs (blood pressure, heart and respiratory rate, and temperature) will be taken after injection. The patient will wait approximately 60 minutes to allow for biodistribution and tumor uptake of the radiopharmaceutical prior to imaging. The patient should void immediately prior (5-10 minutes) to image acquisition. Vital signs (blood pressure, heart and respiratory rate, and temperature) will be taken after the PET/MRI acquisition is complete.

All adverse events occurring within a 24-hour period post-FFNP infusion will be recorded (a phone call to the participant 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.

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 have the option to 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.

5.5.4 Imaging Quality Assurance (QA)/Quality Control (QC) Procedure

QA/QC procedures will include review of DICOM files against study protocols. The PET/MRI scanners must be kept calibrated in accordance

with the manufacturer's recommendations. The scanners should routinely be assessed for quantitative integrity and stability by being tested using current 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 the 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 Research 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 breast PET/MRI will be acquired using 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 T1-weighted imaging without fat suppression and 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. The standard MR-based attenuation correction algorithm provided by GE will be used for this study. The MRI sequence(s) will be co-registered to the PET data for motion correction. Breast specific PET emission data will be acquired simultaneous with the MR images (Figure 1). The entire examination is expected to take 30 – 40 minutes.

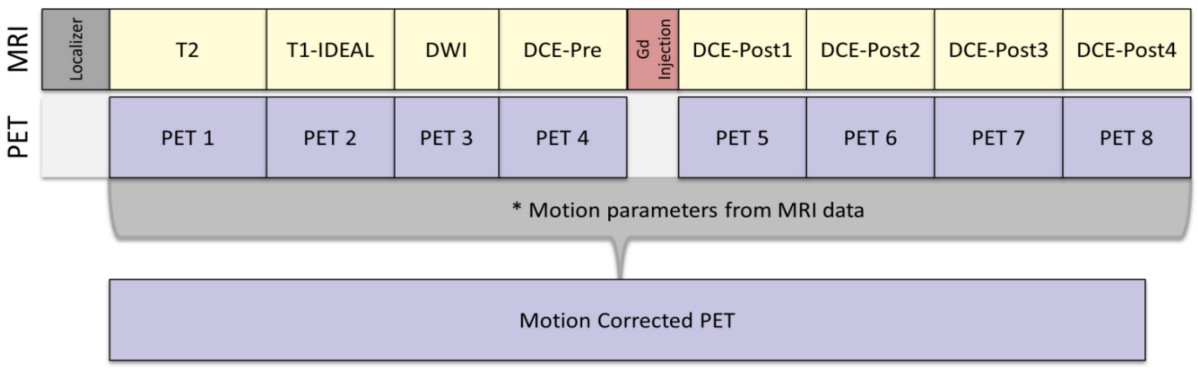


Figure 1: Simultaneous acquisition of PET events during the acquisition of the MRI sequences. PET data will be segmented and motion corrected corresponding to the anatomic position as determined by the MRI sequence coinciding with the PET data acquisition.

5.5.6 Research Imaging Analysis

Two independent readers blinded to the clinical outcome will undergo two sessions of image interpretation of FFNP-PET/MRI scans separated in time by a washout period of at least 4 weeks. Images will be reviewed for general quality. The known biopsy-proven breast cancer(s) will be identified on the PET/MRI examinations 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 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. Each lesion will be analyzed for (1) qualitative appearance (visible above background or not) and (2) semi-quantitative FFNP uptake.

Qualitative Analysis of FFNP uptake (lesion-level): FFNP uptake will be evaluated qualitatively with the following grading scale: no uptake (tumor < background), minimal uptake (tumor = background), mild (tumor slightly > background), moderate uptake (tumor >> background), and intense uptake (tumor >>> background). Tumor uptake will also be dichotomized as increased (moderate or intense uptake) or absent (no uptake, minimal or mild uptake).

Quantitative Analysis of FFNP uptake (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 anatomic imaging. SUV analyses will include, but not be limited to, SUV_{max} (maximum value), SUV_{peak} (small fixed-size ROI centered on most intense part of tumor), SUV_{mean} (average value), and SUV_{sd} (standard deviation). Tumor-to-normal tissue uptake ratios will also be measured. Other PET imaging parameters will include FTV (functional tumor volume; i.e. the volume of tumor tissue with increased FFNP uptake) and TLU (total lesion uptake; i.e. the product of FTV × SUV_{mean}). Within lesion heterogeneity will be explored using the method described by Kurland et al (*16*).

5.6 Clinical Laboratory Testing

A blood sample is to be obtained at the time of imaging (drawn prior to FFNP injection) and submitted for hormonal analysis (progesterone and 17β-estradiol) by the UW Health clinical laboratory. Results from UW Health clinical laboratory automatically post to the subjects' medical records.

5.7 Research-Based Oncotype DX Assay

A genomics-based recurrence risk score will be calculated using a research version of the Oncotype DX test (*17*). The risk score (0-100) is generated from

expression levels of sixteen cancer related genes and five reference genes (**Table 3**) (*18,19*). Scores are further categorized as low-risk (0-17), intermediate-risk (18-30), and high-risk (31-100). 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, if sufficient remaining tissue is available. The Fowler lab will then perform quantitative polymerase chain reactions published by Cronin et al (*20*) and will follow the algorithm of Habel et al (*21*) to calculate the genomics-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: 21 gene panel included in the Oncotype DX Score

HER2 Group	ER Group	Proliferation Group	Invasion Group	Other	Reference
GRB7	ER	Ki67	Stromelysin 3	GSTM1	ACTB
HER2	PGR	STK15	Cathepsin L2	CD68	GAPDH
	Bcl2	Survivin		BAG1	RPLP0
	SCUBE2	Cyclin B1			GUS
		MYBL2			TFRC

5.8 Reproducibility Research Imaging Session (Optional)

Up to 5 PR+ patients who agree to participate in this optional sub-study of the trial will undergo a second (test-rest) study. The test re-test 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 5 PR+ patients onto the sub-study.

5.9 Follow-Up

Adverse events occurring within 24 hours after FFNP infusion will be recorded by the study coordinator who will contact the subject within 1 to 3 days after FFNP administration.

Diagnosis of breast cancer recurrence, established per routine clinical care, will be recorded at the conclusion of the study for those patients with follow-up information available through the electronic medical record system (minimum 1 year after primary breast surgery).

Definition of disease recurrence during follow-up will be as follows:

- **Local recurrence:** histologic evidence of ductal carcinoma in situ or invasive breast cancer in the ipsilateral breast or chest wall.
- **Regional recurrence:** cytologic or histologic evidence of disease in the ipsilateral internal mammary, ipsilateral supraclavicular, ipsilateral

infraclavicular and/or ipsilateral axillary nodes or soft tissue of the ipsilateral axilla.

- **Distant recurrence:** cytologic, histologic, and/or radiographic evidence of disease in the skin, subcutaneous tissue, lymph nodes (other than local or regional metastasis), lung, bone narrow, central nervous system or histologic and/or radiographic evidence of skeletal or liver metastasis.

6. REGISTRATION PROCEDURES

Patients must not start protocol procedures prior to registration. Patients must complete the research breast imaging session prior to any interventions prompted by the clinical breast MRI, if performed prior to the research study, and prior to surgical/oncologic intervention for their biopsy-proven breast cancer as part of their routine clinical care.

6.1 Patient Recruitment

Potential subjects will be identified by the breast imaging radiologist(s) or study team members by reviewing Department of Radiology schedules or electronic medical records of patients with orders for, scheduled for, or having recently completed a clinical breast MRI scan. The study coordinator will contact these patients by phone prior to or after their scheduled MRI and outline the research protocol to the patient.

We also plan to use MyChart, UW Health's patient portal, to recruit patients that appear to meet eligibility criteria. We will obtain all needed approvals from the UW Health and the Clinical Research Data Service and follow the required workflows. We have an OnCore study record that is interfaced with HealthLink and will track individual level recruitment efforts. Once approvals are in place, the IRB approved invitation will be posted to invited participants' research studies page in MyChart. Patients will be provided with instructions on how to either contact the research team or opt out of participation.

For patients 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, the research visit will be scheduled.

The consent process will occur prior to the administration of research procedures. To better accommodate patient schedules, consent procedures will be conducted by phone, when possible. 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.
- 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 Health Insurance Portability and Accountability Act (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:

- The patient does not complete the PET/MRI examination per study protocol
- Pregnancy
- The subject withdraws consent
- Exclusion criteria are discovered after registration
- The patient has a contrast reaction to the gadolinium based contrast agent utilized for the clinical breast MRI

7. TREATMENT/INTERVENTION PLAN

The results of the research breast imaging studies will not be used to direct 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 FFNP for PET imaging.

7.1 Administration Schedule

For each research breast imaging session, patients will receive:

- FFNP, approximately 7 mCi (259 MBq), IV slow infusion over approximately 2 minutes followed by saline flush, once
- Gadolinum based intravenous contrast agent, weight-adjusted dose followed by a saline flush, once

7.2 Dose Modifications

The dose of FFNP is based on radiation dosimetry estimates and on prior published work (*11*). Due to the potential of a reduced radiosynthetic yield or unavoidable time delays, the administered dose may be adjusted at the discretion of the lead clinical investigator, who also serves as the authorized user, based on whether clinically acceptable images can be acquired with the dose administered. Based on prior work, the administered dose of FFNP will be at least 3 mCi but not exceed 10 mCi (*11*). Any such modifications of the agent infusion will be recorded.

7.3 Supportive 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 FFNP, 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 FFNP, 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 FFNP infusion will be recorded by the study coordinator who will contact the subject within 1 to 3 days after FFNP administration.
Standard of care patient management will occur after the FFNP breast PET/MRI examination(s). 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.

Diagnosis of breast cancer recurrence, established per routine clinical care, will be recorded at the conclusion of the study for those patients with follow-up information available through the electronic medical record system (minimum 1 year after primary breast surgery).

8. ADVERSE EVENTS

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

To date, no significant adverse events have been reported as a result of the intravenous (IV) administration of FFNP for PET imaging applications (*11,12*).

As with any IV administered agent, FFNP 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 FFNP, 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, FFNP is a radiopharmaceutical that decays with positron emission. As such, it poses an intrinsic radiation exposure risk. The organ and total body doses associated with FFNP-PET imaging are comparable to or lower than those associated with other widely used clinical nuclear medicine procedures.

FFNP 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. MEASURMENT OF TREATMENT OR INTERVENTION EFFECT

Not applicable since the research imaging studies 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 a table in 5.3.

11. DRUG FORMULATION AND PROCUREMENT

11.1 Gadolinium based intravenous contrast agent

A gadolinium based intravenous contrast agent will be used for the MRI portion of this study. It will be obtained from a commercial source and will be administered following current clinical practice in use at UW Health from breast MR imaging.

11.2 21-[18F]Fluoro-16α,17α–[(R)-1'-α-furylmethylidene) dioxyl]-19-norpregn-4-ene-3,20-dione (FFNP)

FFNP is an investigational new drug which will be used for this study. The radioisotope for FFNP, Fluorine-18, decays by positron emission and has a half-life of 110 minutes. Fluorine-18 is the most frequently used radioisotope for PET radiopharmaceuticals.

11.2.1 Other Names

[F-18]FFNP, FFNP

11.2.2 Classification

Investigational new drug: Radiopharmaceutical/radiotracer

11.2.3 Mode of Action

FFNP binds to progesterone receptor (PR) with high affinity and selectivity (*7-9*). In primary and metastatic breast cancer, uptake of FFNP measured by PET/CT imaging has been shown to correlate with PR expression in biopsy material assayed by immunohistochemistry (*11*). FFNP is primarily cleared from the body via hepatobiliary elimination.

11.2.4 Storage and Stability

In accordance with regulations, the facility conducts several quality control tests on the FFNP 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 FFNP will be approximately 7 mCi (259 MBq) with a specific activity greater than 200 Ci/mmol. Maximum injection dose for a single PET scan will be 10 mCi or less. In all cases, less than 7 µg mass dose of FFNP should be injected, which is a subpharmacologic, “tracer” amount.

FFNP is the only active ingredient. There is no evidence that nonradioactive and radioactive FFNP molecules display different biochemical behavior.

11.2.6 Preparation

Fluorine-18 labeled FFNP is synthesized with high specific activity so the quantity of progestogenic material injected with the radiopharmaceutical is < 7 µg (<16.3 nmol). The final FFNP product solution contains 90% (v:v) USP 0.9% sodium chloride injection and 10% (v:v) USP ethanol alcohol injection. Up to 10 mL of FFNP may be administered for a single PET scan.

Specific manufacturing processes will follow our approved Drug Master File for FFNP.

11.2.7 Route of Administration

Intravenous (IV)

11.2.8 Incompatibilities

N/A

11.2.9 Availability

cGMP-grade FFNP 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 clinical trials using other investigational radiopharmaceuticals, such as ^{18}F-fluoroestradiol and ^{18}F-fluorothymidine. The investigational pharmacist or qualified nuclear medicine technologist will be the responsible party designated by the Investigator.

FFNP is not currently commercially available.

11.2.10 Side Effects

See Section 8.4 for side effects. The radiation effective dose equivalent to the whole body from intravenously injected FFNP is estimated to be 0.02 mSv/MBq (*11*). The dose-limiting organ is the gall bladder, with an average absorbed dose of 0.113 mGy/MBq. The average absorbed dose to the breasts is 0.010 mGy/MBq and the whole-body dose is 0.015 mGy/MBq. The organ and total body doses associated with FFNP 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

Lactating patients are excluded from this study.

Standard safety precautions required when handling radioactive materials (predominantly during the injection and uptake period) should be followed. FFNP requirements are similar to those used for other PET tracers.

12. STATISTICAL CONSIDERATIONS

12.1 Primary Objective

12.1.1 To compare FFNP uptake of biopsy-proven primary PR+ breast malignancies measured using PET/MRI with the reference standard of PR immunohistochemistry (IHC).

The primary objective is to compare FFNP SUV_{max} from PET/MRI with the PR immunohistochemistry semi-quantitative score obtained by using the Allred score (0-8) (*1*). The correlation of the two measures will be evaluated with Pearson's correlation coefficient. The null hypothesis is H_0: p_0=0.20 and the alternative hypothesis is H_1: p_1 =0.70.

A sample size of 21 patients achieves 80% power to detect this difference, using a two-sided hypothesis test with a Type I error of 5%, accounting for 1 dropout. Sample size calculations were done in PASS 14 (NCSS, LLC, Kaysville, Utah. http://www.ncss.com). .

Other measures of FFNP uptake including SUV_{peak} (small fixed-size ROI centered on most metabolically active part of tumor), SUV_{mean} (average value), tumor-to-normal tissue uptake ratio, and total lesion uptake (functional tumor volume × SUV_{mean}) will also be correlated with the PR immunohistochemistry semi-quantitative score obtained by using the Allred score.

12.2 Secondary Objectives

12.2.1 To determine the test-retest reproducibility of quantitative assessment of tumor FFNP uptake.

The reproducibility of the two measurements of FFNP uptake will be evaluated in the 5 subjects who elect to undergo a second imaging session, using summary statistics of tumor FFNP uptake for each reading for PET/MRI. The analysis will be done separately for each reader.

12.2.2 To determine the intra- and inter-observer variability of quantitative assessment of FFNP.

The intra- and inter-reader agreement of SUV values for tumor FFNP uptake will be analyzed with Bland-Altman plots and 95% limits of agreement. Analyses will be conducted on a per-lesion basis, and repeat tumors within the same patient will be assumed to be independent.

12.2.3 To evaluate the optimal cut-point of FFNP uptake for distinguishing between PR negative and PR positive invasive breast cancer.

Receiver operating characteristic (ROC) curve analysis will be performed to determine the optimal cut-point for FFNP uptake to distinguish PR-positive from PR-negative invasive breast cancer, as

defined by the clinical pathology report. The area under the curve 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 FFNP uptake value with the maximum sensitivity and specificity. The analysis will be done separately for each reader.

12.2.4 To estimate the association of tumor FFNP uptake (continuous SUV_{max}) with research-based Oncotype DX scores (0-100).

Scatter plots of continuous FFNP uptake (SUVmax) on the y-axis and research-based Oncotype DX 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 FFNP uptake and research-based Oncotype DX score. The correlation coefficient (rho) and 95% confidence interval will be reported.

12.3 Exploratory Objectives

12.3.1 To evaluate heterogeneity of tumor FFNP uptake.

The coefficient of variation (COV), defined as standard deviation of the standardized uptake value (SUV) and the mean value of the SUV will be assessed. Other descriptive measures such as skewness and kurtosis, which are measures of asymmetry and heaviness of the tails, relative to the normal distribution will be assessed. The analysis will be done separately for each reader.

12.3.2 To correlate tumor FFNP uptake with serum progesterone and estradiol levels.

A correlation analysis of serum progesterone and estradiol levels with tumor FFNP uptake measured with SUV values will be performed using Pearson's or Spearman's rank correlation. Scatter plots, correlation coefficients (rho), 95% confidence intervals, and p values will be reported. This analysis will be done separately for each reader.

12.3.3 To correlate tumor FFNP uptake with disease recurrence.

If there is sufficient follow-up data for disease recurrence, the Kaplan-Meier method will be used to analyze time to disease recurrence, defined as date of imaging day until disease recurrence.

Patients who do not experience disease recurrence will be censored at the date of last available follow-up. A Cox proportional hazards model will be used to evaluate the association of tumor FFNP uptake with time to disease recurrence. If there is insufficient follow-up data, descriptive statistics will be used to summarize the tumor FFNP uptake for those patients with disease recurrence.

12.4 Evaluation of Toxicity

Subjects will be evaluable for toxicity from the time of injection of FFNP until 24 hours after injection. For this evaluation, the study coordinator will contact the subject within 1 to 3 days after FFNP administration.

13. PATHOLOGY REVIEW

13.1 Justification

The primary objective is to measure FFNP uptake of biopsy-proven primary PR+ invasive breast malignancies using PET/MRI. The reference standard will be PR expression measured with immunohistochemistry.

The pathology of the biopsy-proven breast cancer will be evaluated per the current standard of care (histologic diagnosis, grade, ER status, PR status, HER2 status, Ki67 score). At UW Health, Ki67 score is routinely reported as a percentage of darkly stained nuclei across the entire tumor represented on the stained slide. At UW Health, PR status is routinely reported as positive or negative and includes a visual estimation of the percentage of cells staining positive as well as intensity of staining. This information will be obtained from the pathology report in the subjects' electronic medical record and will be used to calculate a semi-quantitative Allred score (*1*). 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 PR IHC slides.

13.2 Required Pathology Materials

Pathology reports will be available through the electronic medical record. Existing pathology slides (PR IHC and H&E) from the breast biopsy used for initial diagnosis will be requested from the Department of Pathology for semi-quantitative 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 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

Requested pathology slides will be sent to the research study pathologists.

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 research imaging 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 UW 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 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 UW Health 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), vitals recorded, medications reviewed, MRI 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 FFNP. 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, FFNP production and administration, and subject compliance and tolerance of research procedures.

The external monitoring plan will be coordinated by the Department of Radiology Medical Imaging Research Support (MIRS) administrative team. MIRS is a working group that provides investigators with the support needed to conduct clinical research that uses imaging as an outcome measure. Members include a core administrative group (physicians, scientists, imaging modality managers, regulatory and administrative support) and ad hoc members recruited when their expertise is required for the review/monitoring of a research project. MIRS administrative team in addition to a 4-person committee of physicians/scientists will meet annually with representatives from the research team, to discuss research progress, AE/SAE reports, as well as other data reports compiled by the study team. If an AE/SAE 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 non-compliance on the part of the study team, and the plan for resolution and a future remediation plan.

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

There has been one “first-in-human” study involving 20 patients using FFNP PET/CT (*11*). There were no adverse events, changes in vital signs, laboratory values or electrocardiograms, or clinically detectable pharmacologic effects. Similarly, there were no adverse events or clinically detectable pharmacological effects associated with FFNP administration in

a subsequent phase 2 study involving 43 patients each receiving 2 FFNP PET/CT scans (*12*).

Risks associated with PET imaging result primarily from the radiation dose from the radiopharmaceutical. For this research study, approximately 7 mCi of FFNP will be administered intravenously which is also the standard dose used for other clinical trials using FFNP (*11,12*). The radiation effective dose equivalent to the whole body from intravenously injected FFNP is estimated to be 0.02 mSv/MBq (*11*). Thus for an administered dose of 7 mCi (259 MBq), the whole-body effective dose equivalent is approximately 5 mSv. The dose-limiting organ is the gall bladder, with an average absorbed dose of 0.113 mGy/MBq. The average absorbed dose to the breasts is 0.010 mGy/MBq and the whole-body dose is 0.015 mGy/MBq. Thus for an administered dose of 7 mCi (259 MBq), the whole-body absorbed dose is approximately 3.885 mGy. 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 current guidance from radiation protection organizations, including American Association of Physicists in Medicine, Health Physics Society, International Organization for Medical Physics, and United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) states that estimates of risk below levels of about 100 mSv are not statistically different from zero. The organ and total body doses associated with FFNP-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.

16.1.3 CT (performed only in rare instances when the 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.

16.1.4 Scanner/Tracer Production Issues

There is a possibility of tracer production issues and scanner malfunction issues. These two issues are rare but not unanticipated. When possible, subjects will simply be rescheduled to a day/time that fits their schedules. If PET tracer has been administered prior to the discovery of a scanner issue, subjects will be paid and withdrawn from this research. The research

team will call the subject within 3 days to inquire about adverse events related to the tracer.

16.1.5 Unexpected findings

All patients in this study will receive the clinical “gold standard”, diagnostic breast MRI and PR measurement via IHC, as part of their clinical care as a requirement for enrollment in the study. Thus, the breast specific PET/MRI research study is not expected to yield any additional information beyond that which was described on the diagnostic exam. Additionally, breast specific PET is not routinely used in clinical practice for local staging and extent of disease and this information will not be used to guide clinical care. For these reasons, incidental findings may not be identifiable (poor quality images) or may not be identified until long after the research exam is performed. Thus, PET images will not be reviewed by a radiologist as a diagnostic exam.

16.1.6 Drug side effects

16.1.6.1 FFNP: See Section 8.4 for possible side effects.

17. REFERENCES

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