Longitudinal Molecular Imaging of Progesterone Receptor Reveals Early Differential Response to Endocrine Therapy in Breast Cancer with an Activating ESR1 Mutation

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Activating mutations in the estrogen receptor (ER) α-gene (ESR1) result in constitutive transcriptional activity in the absence of estrogen and are associated with endocrine resistance in metastatic ER-positive (+) breast cancer. It is not known how activating ESR1 mutations may alter the predictive values of molecular imaging agents for endocrine therapy response. This study investigated the effect of an activating ESR1 mutation on pretreatment ¹⁸F-fluorostriadiol (¹⁸F-FES) uptake and early assessment of endocrine therapy response using ¹⁸F-FDG and ¹⁸F-fluorofuranylnorprogesterone (¹⁸F-FFNP) PET/CT imaging of tumor glucose metabolism and progesterone receptor (PR) expression, respectively. Methods: ER+, PR+ T47D breast cancer cells expressing wild-type (WT) ER or an activating ESR1 mutation, Y537S-ER, were used to generate tumor xenografts in ovariectomized female immunodeficient mice supplemented with 17β-estradiol. Tumor growth curves were determined in the presence or absence of estrogen and for ethanol vehicle control or fulvestrant treatment, a selective ER degrader. Pretreatment ¹⁸F-FES uptake was compared between Y537S-ER and WT-ER tumors. Longitudinal PET/CT imaging with ¹⁸F-FFNP and ¹⁸F-FDG was performed before and 7–9 d after the start of endocrine therapy with fulvestrant. Radiopharmaceutical uptake in Y537S-ER and WT-ER tumors was compared between baseline and follow-up scans. Statistical significance was determined using paired t testing for longitudinal imaging and 2-way ANOVA for the ¹⁸F-FFNP tissue biodistribution assay. Results: Y537S-ER xenografts showed estrogen-independent growth, whereas WT-ER tumors grew only with estrogen. Fulvestrant treatment for 28 d significantly reduced tumor volumes for WT-ER but only stabilized volumes for Y537S-ER. Baseline ¹⁸F-FES uptake did not significantly differ between WT-ER and Y537S-ER tumors. Fulvestrant treatment induced a similar early metabolic response for both WT-ER and Y537S-ER tumors. ¹⁸F-FFNP uptake in WT-ER tumors was significantly reduced after 7 d of fulvestrant treatment; however, this reduction did not occur in Y537S-ER tumors, which showed no significant change between baseline and follow-up PET/CT. Conclusion: Molecular imaging of PR expression dynamics could be a noninvasive approach for early identification of reduced effectiveness of endocrine therapy resulting from activating ESR1 mutations.

Received May 16, 2020; revision accepted Jul. 20, 2020.
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Published online Aug. 28, 2020.
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Estrogen receptor (ER) and progesterone receptor (PR) are expressed in the majority of breast cancer cases and are prognostic and predictive biomarkers. Immunohistochemical assessment of these biomarkers is performed clinically to determine whether endocrine therapy should be used. Patients with metastatic ER-positive (+) or PR+ breast cancer can be treated with various endocrine therapy agents such as aromatase inhibitors or selective ER modulators such as tamoxifen. Selective ER degraders are another option; these are pure ER antagonists, competitively inhibiting ER binding with estrogen and targeting ER protein for proteasome-mediated degradation. Fulvestrant is the only Food and Drug Administration–approved drug in this class for the treatment of ER+ metastatic breast cancer. Although most patients with metastatic ER+ breast cancer have a favorable response to endocrine therapy initially, the response rate decreases with subsequent lines of therapy, indicating the development of resistance (1).

Up to 40% of patients with metastatic ER+ breast cancer treated with endocrine therapy have acquired ER α-gene (ESR1) somatic mutations, which correlate with reduced survival (2,3). Most ESR1 mutations occur at amino acids 537 and 538 in the ligand-binding domain (4). These mutations mimic an agonist-bound receptor conformation, resulting in constitutive transcriptional activity in the absence of estrogen (5,6). Of the reported mutations, tyrosine-537-serine (Y537S) has a high prevalence, has maximal estrogen-independent transcriptional activity, and is more resistant to ER antagonists (7,8). The conformational change in the ligand-binding domain also impacts ligand-binding affinity, with reduced binding of Y537S-ER to estradiol and fulvestrant compared with wild-type (WT) ER (4,7). Higher doses of fulvestrant are required to inhibit transcriptional activity and cell proliferation in Y537S-ER than in WT ER (7–11). Emergence of ESR1 tumor mutations driving endocrine resistance is a significant problem because there are currently no clinically approved therapies that target ESR1 mutations.

Clinical studies of imaging biomarkers to predict response to endocrine therapy have focused primarily on ER using ¹⁸F-fluorostriadiol (¹⁸F-FES) and glucose metabolism using ¹⁸F-FDG (12–18).
Preclinical studies have also identified 18F-fluorofuranylnorprogesterone. 18F-FFNP, a radiolabeled progestin analog that binds to PR, as a potential biomarker of estrogen sensitivity and endocrine therapy response (19–21). PR is a downstream target of activated ER and an indicator of ER functionality such that when ER transcriptional function is blocked, PR protein expression decreases. A study investigating 18F-FFNP PET imaging in breast cancer has demonstrated its safety, dosimetry, and correlation between uptake and tumor PR status, thus supporting its translational potential (22).

It is not known how activating ESR1 mutations in ER+ metastatic breast cancer may alter the predictive values of molecular imaging agents for endocrine therapy response. In this study, we investigated how expression of Y537S-ESR1 impacts the predictive values of 18F-FES, 18F-FDG, and 18F-FFNP. Given the constitutive transcriptional activity and reduced estrogen sensitivity of the mutant receptor, we hypothesized that suppression of 18F-FFNP uptake and inhibition of tumor glycolytic activity will be impaired in tumors expressing Y537S-ER treated with fulvestrant.

MATERIALS AND METHODS

Cell Culture

The experiments adhered to a protocol approved by the Office of Biologic Safety, CRISPR-Cas9 genome-edited T47D cells with WT and ESR1 knock-in of Y537S were kindly provided by Dr. Steffi Oesterreich (University of Pittsburgh) (10) and tested negatively for murine pathogens and Mycoplasma (IDEXX BioResearch). Cells were cultured in RPMI medium (Corning) with 10% fetal bovine serum (VWR) and 1% penicillin/streptomycin (Gibco) at 37°C and 5% CO2. Steroid hormone–depleted conditions consisted of 10% charcoal/dextran–stripped fetal bovine serum in phenol red-free medium with 1% penicillin/streptomycin and 2% l-glutamine.

Droplet Digital Polymerase Chain Reaction (ddPCR)

Reactions were prepared with 25 ng of extracted DNA (DNeasy Blood and Tissue Kit; Qiagen) in ddPCR supermix for probes (Bio-Rad). The forward ESR1 primer was 5′-GGACATGGACATCTGACGTGAG-3′; the reverse primer was 5′-CAAGTGCGTTGTGCGTC-3′. The WT-ESR1 probe was 5′-HXC/CCCTCTATGACCTGCTGC-3′. The Y537S-ESR1 probe was 5′-56-FAM/CCCTCTTGTACCTGCTGC/3′ABFQ-3′ (Integrated DNA Technologies). Droplets were generated with a Bio-Rad QX200 Droplet Generator using a 20-μL reaction mix with 70 μL of droplet generation oil. Droplets were then moved into a 96-well PCR plate and run on a C1000 Thermal Cycler (95°C×10 min, 40 cycles of 94°C×30 s and 60°C×1 min, 98°C×10 min with a 105°C heated lid). PCR products were then subjected to flow cytometry using a QX200 Droplet Reader, measuring the equivalent of 10,000 genomic events. Mutation allele fractions were determined using QuantaSoft Analysis Pro, version 1.0.596 (Bio-Rad).

Mice, Tumor Xenografts, and Treatments

Animal studies adhered to American Association for Laboratory Animal Science guidelines and followed a protocol approved by the Institutional Animal Care and Use Committee. Seven- to 10-wk-old female immunodeficient athymic nude mice (NCR-nu/nu; Charles River) were used for all experiments except for the 18F-FES tissue biodistribution assay, which used NOD scid γ (University of Wisconsin–Madison, Biotron Laboratory Breeding Core). Cells (3,000,000) were injected into the second thoracic mammary fat pads at a 1:1 ratio of Matrigel (BD Biosciences) and phosphate-buffered saline for a total volume of 100 μL. Tumors were measured using calipers, and volume was calculated (lenghth × width2)/2.

To assess estrogen-dependent tumor growth, ovariectomized mice were given a 10 μg/mL concentration of 17β-estradiol (E2) in the drinking water or regular drinking water (19). For fulvestrant treatment studies, ovariectomized mice were subcutaneously implanted with silastic tubing (1.98 mm inner diameter, 3.17 mm outer diameter, 1.6-cm length; Dow Corning) containing 20 μg of E2 (60-d release) to support tumor growth. Mice with palpable tumors (>3-mm diameter) were randomized to control and treatment groups. After randomization, we confirmed for each experiment that there was no significant difference in tumor volume between treatment groups. The mice received subcutaneous twice-weekly injections of fulvestrant (4 mg/ mouse; Sandoz) or vehicle control (100 μL of sunflower oil with ethanol).

Radiopharmaceuticals, Tissue Biodistribution, and Imaging

18F-FES and 18F-FFNP were synthesized by the University of Wisconsin–Madison Radiopharmaceutical Production Facility (23). 18F-FDG was obtained commercially (Sofie). The molar activity of 18F-FES ranged from 86 to 475 GBq/μmol, and 18F-FFNP was 200 GBq/μmol at the end of synthesis. 18F-FES and 18F-FFNP tissue biodistribution assays were performed 1 h after tail vein injection. The injected doses (mean ± SD) of 18F-FES and 18F-FFNP were 1.14 ± 0.04 MBq (~30 μCi) and 3.17 ± 0.17 MBq (~85 μCi), respectively. Activity in tissues was measured using a γ-counter, and data were background-corrected to calculate the percentage injected dose per gram (%ID/g). Tumor-to-muscle ratio was calculated as the ratio of %ID/g for tumor to averaged %ID/g for left and right quadriceps.

18F-FFNP PET/CT was performed at baseline and after 7 d of fulvestrant treatment. To administer equimolar amounts of 18F-FFNP, we injected the mice via the tail vein with an average of 3.87 ± 0.12 MBq (~104 μCi) for baseline imaging and 8.70 ± 0.35 MBq (~235 μCi) for follow-up imaging. For 18F-FDG PET/CT, fasting mice were injected via the tail vein with 5.77 ± 0.20 MBq (~156 μCi) at baseline and after 9 d of fulvestrant. The mice were anesthetized with 2% isoflurane and scanned supine in the small-animal PET/CT scanner (Inveon; Siemens Preclinical Solutions) 1 h after injection. The mice that were to undergo 18F-FDG PET/CT remained anesthetized during the 1-h uptake time. Scanning and reconstruction parameters were reported previously (24). Volumes of interest were drawn around the tumors, around the pituitary gland as an internal positive control for estrogen-regulated PR expression (25), and within the quadriceps as nontarget tissue uptake. Quantitative uptake was expressed as maximum %ID/g.

Histology

Excised tumors were fixed in 10% formalin, embedded in paraffin, and sectioned for staining. The slides were deparaffinized, followed by heat epitope retrieval in citrate buffer (pH 6.0) for 60 min at 95°C. Immunostaining was performed for PR (1:100 NCL-L-PGR-312; Leica Biosystems) and ER (1:100 SP1; Thermo Fisher) using the Vectastain ABC HRP Kit (Vector Laboratories). An experienced breast pathologist masked to treatment group evaluated the percentage of tumor cells with positive staining and the intensity of the staining (none, weak, moderate, or strong).

Statistical Analysis

For the longitudinal PET/CT studies, paired t tests were used to assess change in radiotracer uptake between the 2 imaging time points for the same tumor within the same mouse. Paired t tests were used to compare 18F-FES uptake between tumor types within the same mouse. Two-way ANOVA with Tukey posttesting was used for analysis of the transcriptional activity results and for the 18F-FFNP tissue biodistribution assay (GraphPad Prism, version 8). Results are presented as mean ± SE. A P value of less than 0.05 was considered significant.
RESULTS

In Vitro Analysis of CRISPR-Edited T47D Y537S-ER and WT-ER Cells

The Y537S mutation allele fraction was 50% in Y537S-ER cells, indicating heterozygous knock-in of the mutation and 0% in WT-ER cells. A 32.7 ± 10.2-fold increase in transcriptional activity was observed in Y537S-ER cells compared with WT-ER in the absence of estrogen (P = 0.0195; Supplemental Fig. 1; supplemental materials are available at http://jnm.snmjournals.org). Constituitive transcriptional activity observed with Y537S-ER is consistent with published studies (8,10,24).

Effect of Estrogen and Endocrine Therapy on WT-ER and Y537S-ER Tumor Growth

Although WT-ER tumor xenografts demonstrated sustained growth only with estrogen, Y537S-ER tumors grew with or without estrogen (Fig. 1A). Furthermore, Y537S-ER tumors without estrogen grew faster than WT-ER tumors with estrogen. Consistent with in vitro transcriptional function, the in vivo growth of Y537S-ER tumor xenografts is also estrogen-independent. The Y537S allele fraction was 52% ± 2.5% and 0.2% ± 0.2% in Y537S-ER and WT-ER tumors, respectively, indicating that the heterozygous presence of the mutation is maintained when these cells are grown as tumor xenografts.

Given previous work demonstrating reduced binding affinity of Y537S-ER for fulvestrant, with higher doses required to inhibit transcriptional activity and proliferation (7–11), we hypothesized that tumors expressing Y537S-ER would be less sensitive to growth inhibition by fulvestrant than would WT-ER. A significant reduction in WT-ER tumor volume was observed by day 10 for fulvestrant-treated mice compared with control mice (P = 0.003) (Fig. 1B), with an approximately 46% total reduction in volume from baseline to the 28-d endpoint. Y537S-ER tumors in the control group continued to increase over time, with an approximately 122% total increase in volume. Y537S-ER tumor growth was arrested with fulvestrant treatment, but tumor volumes did not decrease. A significant difference in Y537S-ER tumor volumes between treatment groups was observed by day 16 (P = 0.004). Thus, tumor growth responses with fulvestrant treatment differed between WT-ER (reduced tumor volumes) and Y537S-ER xenografts (stable tumor volumes), indicating that Y537S-ER tumors are less sensitive to growth inhibition by fulvestrant than are WT-ER.

Predictive Value of Baseline 18F-FES Uptake for Therapy Response

Several studies have demonstrated 18F-FES PET imaging as a potential predictive biomarker for endocrine therapy response in patients with metastatic ER+ breast cancer (13,18,26). Thus, we examined whether differences in baseline 18F-FES uptake exist that could predict the differential growth response to fulvestrant between tumor types. There was no significant difference in 18F-FES uptake between Y537S-ER and WT-ER tumors (Fig. 2A). The tumor-to-muscle ratio was 4.56 ± 0.33 for Y537S-ER tumors and 4.21 ± 0.44 for WT-ER tumors (P = 0.2772) (Fig. 2B). Uterus uptake was 7.89 ± 1.01 %ID/g. Thus, comparable baseline 18F-FES uptake in Y537S-ER and WT-ER tumors does not provide insight into their different growth responses to fulvestrant treatment.

Longitudinal 18F-FDG PET/CT Assessment of Therapy Response

Determination of metabolic response by 18F-FDG PET/CT has been shown in small clinical studies to be a potential biomarker of endocrine therapy response (12,15,16,27). Thus, we investigated whether differences in metabolic response exist between Y537S-ER and WT-ER tumors that correspond to the differential growth response to fulvestrant treatment. 18F-FDG PET/CT of mice bearing WT-ER and Y537S-ER xenografts was performed before and after 9 d of fulvestrant treatment, a time point before statistically significant changes in tumor size. Between the baseline and follow-up scans, 18F-FDG uptake decreased for both tumor types (Fig. 3; Supplemental Table 1). Reduction in posttreatment 18F-FDG uptake was −31.85% ± 8.18% for WT-ER tumors and −28.29% ± 7.69% for Y537S-ER tumors (P = 0.7735). These data indicate that fulvestrant treatment induces a similar metabolic response in WT-ER and Y537S-ER tumors despite ultimately distinct growth responses.

Longitudinal 18F-FFNP PET/CT Assessment of Therapy Response

We hypothesized that suppression of 18F-FFNP uptake would be greater in tumors expressing WT-ER than in Y537S-ER in response to fulvestrant, because of the reduced binding affinity of Y537S-ER for fulvestrant and reduced treatment efficacy for inhibiting Y537S-ER transcriptional activity, as previously reported (7–11). 18F-FFNP PET/CT of mice bearing WT-ER and Y537S-ER tumor xenografts was performed before and after 7 d of fulvestrant treatment (Fig. 4; Supplemental Table 2). For WT-ER tumors, 18F-FFNP uptake decreased from 3.97 ± 0.35 %ID/g at baseline to 2.10 ± 0.20 %ID/g (P = 0.0001) on the follow-up scan. However, there was no significant change in 18F-FFNP uptake for Y537S-ER tumors between the baseline (4.18 ± 0.37 %ID/g) and follow-up (3.92 ± 0.5 %ID/g) scans (P = 0.3326). Reduction in posttreatment 18F-FFNP uptake was −47.86% ± 2.60% for WT-ER tumors and −7.04% ± 8.07%
for Y537S-ER tumors ($P = 0.0033$). As a positive control for fulvestrant inhibition of ER-regulated PR expression, $^{18}$F-FFNP uptake in the pituitary was reduced to $2.07 \pm 0.04 \% \text{ID/g}$ during treatment, compared with $3.05 \pm 0.08 \% \text{ID/g}$ at baseline ($P = 0.0003$) (Supplemental Fig. 2).

To independently confirm these results, we used a separate cohort of mice bearing Y537S-ER or WT-ER tumor xenografts for a tissue biodistribution assay in which $^{18}$F-FFNP uptake was directly measured in excised tumors. For WT-ER tumor–bearing mice treated with fulvestrant for 7 d, $^{18}$F-FFNP uptake was less than in the vehicle control group ($1.10 \pm 0.08 \% \text{ID/g}$ vs. $3.75 \pm 0.35 \% \text{ID/g}$, $P = 0.0008$) (Fig. 5). As with the PET/CT results, there was no significant difference in $^{18}$F-FFNP uptake in Y537S-ER tumors between the fulvestrant and control groups ($2.68 \pm 0.13 \% \text{ID/g}$ vs. $3.24 \pm 0.35 \% \text{ID/g}$, $P = 0.8021$). As a positive control, $^{18}$F-FFNP uptake in the uterus was lower in the fulvestrant group than in the control group for both Y537S-ER tumor–bearing mice ($P = 0.0011$) and WT-ER tumor–bearing mice ($P < 0.0001$). Excised uteri weighed less in fulvestrant-treated mice than in control mice (WT-ER tumor–bearing mice: $50 \pm 8$ vs. $155 \pm 25$ mg, $P = 0.0002$; Y537S-ER tumor–bearing mice: $60 \pm 4$ mg vs. $193 \pm 10$ mg, $P = 0.0001$), confirming appropriate dosing for ER antagonism.

The PR immunohistochemistry results agreed with $^{18}$F-FFNP tumor uptake. A decreased percentage of PR-positive cells and reduced staining intensity was observed in WT-ER tumors treated with fulvestrant but not in Y537S-ER tumors (Supplemental Table 3; Supplemental Fig. 3).

**DISCUSSION**

The purpose of this study was to determine how altered ER signaling caused by an activating $ESR1$ mutation affects the prediction and early assessment of endocrine therapy response using molecular imaging. We demonstrated distinct growth phenotypes for tumor xenografts expressing Y537S-ER and WT-ER treated with estrogen or fulvestrant endocrine therapy. As expected, WT-ER tumors were strictly estrogen-dependent for growth, with reduced tumor volumes in response to fulvestrant. In contrast, Y537S-ER tumors did not require estrogen for growth and were less sensitive to growth inhibition by fulvestrant than were WT-ER tumors. Baseline $^{18}$F-FES uptake did not significantly differ between Y537S-ER and WT-ER tumors. Likewise, early metabolic response was similar between the 2 tumor types, with decreased $^{18}$F-FDG uptake in response to fulvestrant. However, $^{18}$F-FFNP uptake decreased in WT-ER tumors but was persistently elevated in Y537S-ER tumors early after the start of fulvestrant treatment. Differences in $^{18}$F-FFNP uptake were observed before changes in tumor size. These results suggest that $^{18}$F-FFNP PET imaging of PR expression
dynamics could be an effective approach for early identification of reduced effectiveness of endocrine therapy resulting from activating ESR1 mutations. The noninvasive approach and ability to assess response across multiple metastatic lesions are advantages of 18F-FFNP PET imaging over the repeated biopsies required for PR immunohistochemistry.

The clinical significance of ESR1 mutations has only recently been recognized, and testing is not yet routinely performed. Thus, the tumor ESR1 mutation status in previous clinical PET imaging studies of ER+ breast cancer is not known, with the exception of one report published last year. Boers et al. investigated the relationship between intertumoral 18F-FES heterogeneity and time to progression in metastatic ER+ breast cancer patients treated with endocrine therapy combined with cyclin-dependent 4/6 kinase inhibition (14). ESR1 mutations were present in circulating tumor DNA from 13 of 23 patients but were not associated with 18F-FES uptake (14).

We have previously shown that measurement of ER ligand binding with 18F-FES is not altered in genetically engineered breast cancer xenografts expressing WT-ER or the constitutively active ESR1 mutations, Y537S and Y537C (24). The triple-negative breast cancer model used in our prior study allowed testing of 18F-FES binding to mutant receptors in isolation from endogenous WT ER protein (24). 18F-FES results from this study using CRISPR-edited T47D cells with heterozygous ESR1 mutation expression agree with our prior observations. Prospective testing for ESR1 mutations in future studies of 18F-FES PET imaging is important to validate these findings, particularly with the recent Food and Drug Administration approval of 18F-FES.

Similar early metabolic responses (reduced 18F-FDG uptake) were observed in both WT-ER and Y537S-ER tumors in response to fulvestrant. This observation was unexpected, in view of our hypothesis that Y537S-ER tumors treated with fulvestrant will have less inhibition of glycolytic activity due to reduced endocrine sensitivity of the mutant receptor. Previous preclinical studies have demonstrated that fulvestrant treatment decreases 18F-FDG uptake in ER+ breast cancer xenografts without known ESR1 mutations. He et al. demonstrated reduced 18F-FDG uptake in ER+ ZR-75-1 tumors after 21 d of fulvestrant treatment when tumor volumes are decreased compared with vehicle control (28).

We also demonstrated that 18F-FDG uptake decreased after 7 and 14 d of fulvestrant treatment in endocrine-sensitive ER-, PR-, STAT1 (signal transducer and activator of transcription 1)-deficient mouse
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manmary tumors, SSM3, but remained unchanged in endocrine-resistant ER+, PR+ SSM2 tumors (20). SSM2 tumors were completely resistant to fulvestrant, with the tumor growth rate being identical to that in ethanol vehicle control tumors—an observation that differs from the growth stabilization phenotype of Y537S-ER tumors observed in this study. Thus, the mechanism and magnitude of endocrine therapy resistance appear to affect the metabolic response pattern observed.

These results suggest that early assessment of PR expression dynamics could indicate insufficient inhibition of ER signaling in tumors with activating ESR1 mutations. Persistent 18F-FFNP uptake by Y537S-ER tumors after 7 d of fulvestrant was distinct from the reduced uptake seen with WT-ER tumors, indicating an inadequate therapy response despite maximal ER antagonist dosing. These results agree with our previous preclinical studies demonstrating how changes in 18F-FFNP uptake reflect a response to endocrine therapy in ER+, PR+ STAT1-deficient tumors and provide predictive information beyond that provided by 18F-FES or 18F-FDG PET imaging (19,20). Collectively, 18F-FFNP appears to be a robust functional imaging biomarker of endocrine sensitivity and may be generalizable to endocrine therapy resistance acquired through different cellular mechanisms. Clinical trials confirming the predictive value of 18F-FFNP PET imaging in patients, such as a recently completed trial at Washington University (NCT02455453), are important for further validation.

The observed Y537S ESR1 allele fraction in clinical samples from patients with metastatic breast cancer typically ranges between 23% and 62%, but can be as low as 4% (8). Thus, the 52% Y537S ESR1 allele fraction present in the tumor model system used in our study appropriately represents the allele fractions found in metastases. However, the utility of serial 18F-FFNP imaging as a biomarker for response to fulvestrant in tumors with low fractions of Y537S ESR1 mutation alleles would need to be directly tested.

This investigation focused on one ESR1 mutation and one endocrine therapy agent. Other activating ESR1 mutations may yield distinct results, since mutation site–specific gene regulation and antiestrogen sensitivity have been recognized (7,10,29). Also, this work assessed 18F-FES uptake before the start of endocrine therapy, similar to an ongoing multicenter clinical trial (NCT02398773). It is possible that differences in residual ER binding capacity during fulvestrant treatment could be observed between tumors expressing activating ESR1 mutations and WT. Imaging of other pathways, such as glutamine metabolism, could also be informative for assessing therapy response, because Y53S-ER cells were recently shown to have glycolytic rates similar to those of WT-ER cells, but with enhanced mitochondrial activity and glutamine use (30).

CONCLUSION

These findings suggest that 18F-FFNP PET imaging is capable of differentiating endocrine therapy effects in breast cancer with activating Y537S ESR1 mutations. Our results also support ESR1 mutation testing in clinical trials involving molecular imaging, since distinct responses can occur with endocrine therapy depending on mutation status.

DISCLOSURE

The University of Wisconsin–Madison Department of Radiology receives research support from GE Healthcare. Services were provided by the Translational Research Initiatives in Pathology laboratory, which is in part supported by the University of Wisconsin Department of Pathology and Laboratory Medicine and UWCCC (grant P30 CA014520), the Experimental Pathology Laboratory (grant P30 CA014520), and the University of Wisconsin–Madison Biophysics Instrumentation Facility, established with support from the University of Wisconsin–Madison and grants BIR-9512577 (NSF) and S10 RR13790 (NIH). Financial support was received from the Clinical and Translational Science Award program, through the NIH National Center for Advancing Translational Sciences (grant UL1TR002373), a University of Wisconsin–Madison Institute of Clinical and Translational Research KL2 Scholar Award (KL2TR000428), an American Cancer Society Institutional Research Grant Pilot Award (IRG-15-213-51), and a Mary Kay Innovative/Translational Cancer Research Grant. No other potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

We thank Dr. Steffi Oesterreich (University of Pittsburgh) for kindly providing cells. We thank the University of Wisconsin–Madison Cyclotron Laboratory for 18F production, the Radiopharmaceutical Production Facility for 18F-FFNP and 18F-FES synthesis, and the University of Wisconsin Small Animal Imaging & Radiotherapy Facility (Cancer Center Support Grant NCI P30 CA014520) for services provided.

KEY POINTS

QUESTION: How do activating ESR1 mutations affect the predictive values of 18F-FES, 18F-FDG, and 18F-FFNP as endocrine therapy response biomarkers in ER+ breast cancer?

PERTINENT FINDINGS: 18F-FFNP uptake decreased in WT-ER tumors but was persistently elevated in Y537S-ER early after the start of fulvestrant treatment.

IMPLICATIONS FOR PATIENT CARE: Early assessment of PR expression dynamics using 18F-FFNP PET imaging could indicate insufficient inhibition of ER signaling and inadequate therapy in tumors with activating ESR1 mutations.

REFERENCES


