Pharmacodynamic Imaging Guides Dosing of a Selective Estrogen Receptor Degrader

Pedram Heidari1, Francis Deng1,2, Shadi A. Esfahani1, Alicia K. Leece1, Timothy M. Shoup3, Neil Vasdev3, and Umar Mahmood1

Abstract

Purpose: Estrogen receptor (ER) targeting is key in management of receptor-positive breast cancer. Currently, there are no methods to optimize anti-ER therapy dosing. This study assesses the use of 16α-[18F]-fluorooestradiol (16α-F-FES) PET for fulvestrant dose optimization in a preclinical ER+ breast cancer model.

Experimental Design: In vitro, 16α-F-FES retention was compared with ERα protein expression (ELISA) and ESR1 mRNA transcription (qPCR) in MCF7 cells (ER+). Therefore, expression of ERα is an important factor in prediction of prognostic and effectiveness of anti-hormone therapy. Patients with ERα+ tumors typically have longer overall survival and are more likely to respond to hormone-targeted therapies (3). The expression of ERα is highly variable among patients with breast cancer and, moreover, there is a high degree of heterogeneity found in the level of ERα expression among the metastatic foci in individual patients (4, 5).

Selective estrogen receptor downregulators (SERD) are a relatively new class of drugs for the treatment of ER-expressing breast cancer. Fulvestrant, the prototypical SERD, competitively binds to and irreversibly inhibits ER activity. Although the mechanism of action is similar to that of anti-ER therapies, some preclinical studies have demonstrated that fulvestrant-induced ER downregulation is dose-dependent (13, 14), the foray into high-dose regimens proceeded empirically, with a deduced assumption of the approved dose was suboptimal (15, 16). The development of a noninvasive approach to quantify how ER level changes over the course of monthly treatment cycles (6–8). Fulvestrant is currently indicated for the treatment of metastatic breast cancer in postmenopausal women. While its efficacy is well-established in first-line and second-line settings, only a fraction of women treated with fulvestrant experience an objective response (9–11). Some hypothesized that the approved dose was suboptimal, spurring trials of different dosing regimens (12). While limited studies have demonstrated that fulvestrant-induced ER downregulation is dose-dependent (13, 14), the foray into high-dose regimens proceeded empirically, with a deduced assumption of the level of ER downregulation with fulvestrant therapy and how ER level changes over the course of monthly treatment cycles (15–18). The development of a noninvasive approach to quantify early pharmacodynamic effects would facilitate dose optimization at both the individual and treatment population levels.

Molecular imaging modalities, such as PET, permit early evaluation of tumor biology and assessment of treatment response, well before tumor morphology or histopathology changes occur (19). The estrogen-based radiopharmaceutical 18F-fluorooestradiol (18F-FES) is a PET imaging agent that has high binding affinity and selectivity for the ERα subtype and has been shown to exhibit high specific uptake by ER-rich target tissues and ERα-positive mammary tumors (20–22). The agent focially accumulates at sites of specific overexpression of ERα. The use of 18F-FES PET as a measure of tumoral ERα expression has been validated in clinical studies (20, 23, 24). 18F-FES imaging, in limited studies, has been shown to be of value in prediction of responsiveness to anti-hormone therapy (25–28). This radiotracer can be used to

Results: In vitro, we observed a parallel graded reduction in 16α-F-FES uptake and ER expression with increased fulvestrant doses, despite enhancement of ER mRNA transcription. In xenografts, ER expression significantly decreased with increased fulvestrant dose, despite similar mRNA expression and Ki67 staining among the treatment groups. We observed a significant dose-dependent reduction of 16α-F-FES PET mean standardized uptake value (SUVmean) with fulvestrant treatment but no significant difference among the treatment groups in 16α-F-FDG PET SUVmean.

Conclusions: We demonstrated that 16α-F-FES uptake mirrors the dose-dependent changes in functional ER expression with fulvestrant resulting in ER degradation and/or blockade; these precede changes in tumor metabolism and proliferation. Quantitative 16α-F-FES PET may be useful for tracking early efficacy of ER blockade/degradation and guiding ER-targeted therapy dosing in patients with breast cancer. Clin Cancer Res; 21(6):1340–7. ©2015 AACR.

Introduction

Estrogen receptor α (ERα) is expressed in the majority of human breast cancers and is a critical driver of breast tumorigenesis (1, 2). Therefore, expression of ERα is an important factor in prediction of prognostic and effectiveness of anti-hormone therapy. Patients with ERα+ tumors typically have longer overall survival and are more likely to respond to hormone-targeted therapies (3). The expression of ERα is highly variable among patients with breast cancer and, moreover, there is a high degree of heterogeneity found in the level of ERα expression among the metastatic foci in individual patients (4, 5).

Selective estrogen receptor downregulators (SERD) are a relatively new class of drugs for the treatment of ER-expressing breast cancer. Fulvestrant, the prototypical SERD, competitively binds
quantitate the ERα density in breast cancer (24, 27) and therefore can be used to precisely monitor the effects of any drug that abolishes ERα production, promotes ERα degradation, or that competes with 18F-FES for ERα binding (ref. 29; Fig. 1). Thus, 18F-FES PET imaging provides an unprecedented opportunity to assess immediate drug efficacy on ER capacity and guide dose selection in individual patients.

The objective in this study was to use the quantitative nature of 18F-FES PET imaging to monitor the ER that is occupied and/or degraded by fulvestrant (SERD) treatment at a given time, based on changes in the levels of available receptor for 18F-FES binding.

Translational Relevance

Fulvestrant, a selective estrogen receptor (ER) degrader, is approved for treatment of ER-positive metastatic breast cancer in postmenopausal women. The optimal dosing has been an area for debate; the lower-than-expected clinical performance in breast cancer may be partially explained by inadequate fulvestrant dosing. This study provides evidence that responsiveness to anti-ER therapies such as fulvestrant could be measured by early pharmacodynamic imaging of ER binding/degradation. We demonstrate that 18F-FES uptake mirrors the dose-dependent changes in functional ER expression with fulvestrant treatment, which precedes the changes in tumor metabolism and proliferation. Therefore, 18F-FES PET could be employed early in treatment to guide improved dosing of anti-ER therapeutics. This technology could also be used in drug development to measure effectiveness at the intended therapeutic targets to help refine patient selection and dosing levels for agents in clinical trials. Moreover, this approach may be employed for other receptor-targeted therapies in cancer treatment.

In vitro 18F-FES retention with variable dose fulvestrant treatment

MCF-7 cells were seeded in 24-well plates at 5 × 10^4 cells per well and were allowed to grow and attach for 24 hours. The subconfluent cultures were serum-starved and incubated for 24 hours with fulvestrant (10^{-12} to 10^{-6} mol/L Sigma-Aldrich) or vehicle only [0.1% DMSO (Sigma-Aldrich) in base culture medium] in triplicate. Each well was incubated with 25 μCi 18F-FES in the same formulation of treatment medium as before, for 30 minutes at 37°C. Wells were gently washed 3 times in cold Hank’s Balanced Salt Solution (HBSS; Fisher Scientific), trypsinized, and transferred to counting tubes. Radiotracer uptake was quantified using a gamma counter (WIZARD2; PerkinElmer). Total counts per well were corrected for decay and normalized by defining the maximum uptake group as 100% and nonspecific uptake by vehicle-treated MDA-MB-231 cells as 0%.

Cellular ER expression and ESR1 mRNA concentration

MCF7 cells were plated in 6-well plates at 2.5 × 10^4 cells per well. The subconfluent cultures were serum-starved for a day and then incubated for 24 hours with fulvestrant (10^{-12} to 10^{-6} mol/L) or vehicle. Wells were washed with Dulbecco PBS without calcium (Fisher Scientific) and trypsinized. Using a PARIS Kit (Ambion, Life Technologies), cellular extract was fractionated into nuclear and cytoplasmic fractions. Absolute nuclear protein concentration was quantified by micro BCA assay (Pierce, Thermo Scientific). All cells were maintained in a humidified incubator at 37°C with 5% CO₂. The cells were in culture for less than 6 months after resuscitation. Cell lines undergo comprehensive quality control and authentication procedures by ATCC before shipment. These include testing for mycoplasma by culture isolation, Hoechst DNA staining, and PCR, together with culture testing for contaminant bacteria, yeast, and fungi. Authentication procedures used include species verification by DNA barcoding and identity verification by DNA profiling.

Materials and Methods

Radiopharmaceuticals

18F-FDG was purchased from IBA Molecular. 16t.18F-FES was synthesized according to the literature (30). The mean specific activity of ¹⁸F-FES used in this study was 6.1 Ci/μmol (range, 5.4–6.8 Ci/μmol).

Cell culture

Cell lines and culture media were obtained from ATCC. The ER-expressing human breast adenocarcinoma cell line, MCF7, and triple-negative human breast adenocarcinoma line, MDA-MB-231, were grown in DMEM and Leibovitz L-15 medium, respectively. Both media were supplemented with 10% FBS (Atlanta Biologicals) and 1% penicillin/streptomycin (Gibco, Life Technologies). All cells were maintained in a humidified incubator at 37°C with 5% CO₂. The cells were in culture for less than 6 months after resuscitation. Cell lines undergo comprehensive quality control and authentication procedures by ATCC before shipment. These include testing for mycoplasma by culture isolation, Hoechst DNA staining, and PCR, together with culture testing for contaminant bacteria, yeast, and fungi. Authentication procedures used include species verification by DNA barcoding and identity verification by DNA profiling.
Fisher Scientific Inc.) against a BSA standard curve, and RNA was quantitated using a ND-1000 spectrophotometer (NanoDrop). Intralsample coefficient of variation of total nuclear protein content was 12.7% (n = 9), indicating relatively little effect of brief fulvestrant treatment on cellularity.

Relative ERex protein expression was measured by sandwich ELISA (Active Motif) using 5 µg nuclear extract per well. Results are reference (600 nm) wavelength-subtracted absorbance, normalized by defining the maximum expression group as 100% and nonsppecific binding by nuclear lysate from MDA-MB-231 cells as 0%. Technical ELISA duplicates were averaged to derive values for biologic replicates in independent experiments.

Relative ESR1 mRNA concentration was measured by quantitative reverse transcription PCR (qRT-PCR). In independent culture experiments, 36 or 200 ng total RNA was used for reverse transcription (High Capacity RNA-to-cDNA kit; Life Technologies). Of the 20 µl cDNA reaction volume for each sample, 4.5 µl was used for TaqMan gene expression assay reaction for human ESR1 (Hs00174860_m1) and GAPDH endogenous control (Life Technologies). Thermocycling was conducted on a 7500 Fast Real-Time PCR system (Applied Biosystems, Life Technologies). Cycle thresholds (Ct) were set automatically in SDS software (version 1.4; Applied Biosystems) and normalized by subtracting the Ct value for GAPDH from that of ESR1 (denoted as ΔCt). Results were reported as ΔCt, so that values relate directly with the logarithm of target mRNA concentrations.

Tumor implantation and treatment

Female athymic nude mice (6–8 weeks old) were obtained from Charles River Laboratories. Animal protocols were approved by the Subcommittee on Research Animal Care at Massachusetts General Hospital (Boston, MA). To reduce competition from the endogenous estradiol, all the mice were ovariectomized at least 1 week before tumor implantation. We used intermittent estradiol dosing to minimize competition with radiotracer at the time of imaging. Mice were supplemented with daily subcutaneous injections of 17β-estradiol [Abcam; 20 µg in 20 µL sesame oil: ethanol (9:1; v:v)] beginning at least 3 days before tumor implantation (31). MCF7 cultures were harvested and resuspended 1:1 (v:v) in Matrigel (BD Biosciences). Approximately 5 × 10⁶ cells in 100 µL were injected subcutaneously over the right upper flank. Tumor growth was monitored by caliper measurements along 2 perpendicular axes. When tumors grew to approximately 5 mm in diameter, mice were randomly allocated to treatment groups. At treatment time, estradiol supplementation was withdrawn. Mice received one subcutaneous injection of fulvestrant (0.05, 0.5, or 5 mg) or vehicle [100 µL sesame oil: ethanol (9:1; v:v)]. Two days following treatment, mice were either imaged with ¹⁸F-FES PET/CT or euthanized to harvest the tumor for analyses. Caliper-measured tumor size did not change significantly between treatment and analysis time points. Across all analyzed mice, average 2-dimensional tumor size measurements using a caliper were 6.42 ± 1.44 × 4.95 ± 0.70 mm (mean ± SD) at treatment time. The tumor dimensions were 6.98 ± 1.61 × 5 ± 1.16 mm, 6.36 ± 2.33 × 4.63 ± 0.81 mm, 6.28 ± 0.60 × 5.16 ± 0.20 mm, and 6.45 ± 1.40 × 5.08 ± 0.42 mm in the vehicle, low-dose (0.05 mg), medium-dose (0.5 mg), and high-dose (0.05 mg) groups, respectively. Tumor volumes as measured on computed tomography (CT) were on average 72 ± 18 mm³.

Small-animal PET

For ¹⁸F-FDG-PET only, animals were fasted for at least 4 hours before radiotracer injection. ¹⁸F-FES and ¹⁸F-FDG were respectively injected 2 and 3 days after treatment (5–6 mice per treatment group) via a 30-gauge catheter introduced in the lateral tail vein. Actual activities injected, calibrated to time of scan, were 9.7 ± 2.2 MBq (263 ± 59 µCi; mean, SD) of ¹⁸F-FES and 9.9 ± 1.7 MBq (268 ± 54 µCi) of ¹⁸F-FDG. Actual times elapsed between injection and PET acquisition were 69 ± 12 minutes (FES) and 69 ± 7 minutes (FDG). PET data were acquired under a whole-body emission protocol on an Argus small-animal PET/CT scanner (Sedecal) for 15 minutes in 2 bed positions. Images were reconstructed with a 2D ordered-subset expectation maximization (2D-OSEM) algorithm. CT data were acquired in 100-µm resolution. The scans were acquired with tube voltage of 40 kVp and tube current of 140 µA. Total scan duration was approximately 14 minutes. Image data were reconstructed using the Feldkamp algorithm. Three-dimensional regions of interest (ROI) were manually drawn around tumors based on co-registered PET/CT scans. Mean standardized uptake values (SUVmean) were calculated within the ROI. ¹⁸F-FES SUVmean was also normalized to blood pool activity at the time of scan from a 3D spherical 3-mm ROI drawn within the heart, and ¹⁸F-FDG uptake was expressed as target-to-background ratio (TBR).

Statistical analyses

Statistical analysis was performed with Prism software (version 5; GraphPad). Data from multiple groups were compared by one-way ANOVA followed by Holm–Sidak post hoc multiple comparisons test. For in vitro experiments only, semilogarithmic dose–response data were fit to 4-parameter sigmoidal curves. Correlation analysis was conducted using Pearson r. Data are graphed as
In vivo experiments. In all experiments, MCF7 cells were treated with fulvestrant for 24 hours. The results were normalized to an ER-negative control cell line, MDA-MB-231. A, 18F-FES retention is plotted against fulvestrant concentration (log (mol/L)). The 18F-FES uptake gradually decreases with increasing dose of fulvestrant (ANOVA; $P = 0.0005$). Data are triplicate samples. Results were radioactive decay-corrected. B, ERα expression determined by sandwich ELISA in MCF7 cells treated with a range of fulvestrant concentrations (log (mol/L)). Data are from biologic replicates in 2 independent experiments and normalized to MDA-MB-231. C, ESR1 mRNA expression versus fulvestrant concentration as determined by qRT-PCR. Results displayed are average cycle threshold ($C_t$) for ESR1 subtracted from that of the housekeeping gene GAPDH for each group. $–ΔC_t$ approximates the base 2 logarithm of relative gene expression. Data are from biologic replicates in 2 independent experiments. Error bars are SEM.

![Figure 2](image-url)

**Figure 2.**

In vivo experiments. In all experiments, MCF7 cells were treated with fulvestrant for 24 hours. The results were normalized to an ER-negative control cell line, MDA-MB-231. A, 18F-FES retention is plotted against fulvestrant concentration (log (mol/L)). The 18F-FES uptake gradually decreases with increasing dose of fulvestrant (ANOVA; $P = 0.0005$). Data are triplicate samples. Results were radioactive decay-corrected. B, ERα expression determined by sandwich ELISA in MCF7 cells treated with a range of fulvestrant concentrations (log (mol/L)). Data are from biologic replicates in 2 independent experiments and normalized to MDA-MB-231. C, ESR1 mRNA expression versus fulvestrant concentration as determined by qRT-PCR. Results displayed are average cycle threshold ($C_t$) for ESR1 subtracted from that of the housekeeping gene GAPDH for each group. $–ΔC_t$ approximates the base 2 logarithm of relative gene expression. Data are from biologic replicates in 2 independent experiments. Error bars are SEM.

Results

**In vitro analysis of MCF7 cells**

We performed 18F-FES retention studies after incubating MCF7 cells with serial dilution doses of fulvestrant for 24 hours. Significant dose-dependent changes in 18F-FES uptake were observed (Fig. 2A). The 50% inhibitory concentration ($IC_{50}$) was 8.2 nmol/L (95% confidence interval (CI), 3.5 × 10^{-10} to 1.9 × 10^{-7} mol/L; model $R^2$: 0.834). Compared with vehicle control, specific 18F-FES retention decreased significantly by 48.3% ($P = 0.03$), 53.1% ($P = 0.02$), 79.7% ($P < 0.001$), and 85.7% ($P < 0.001$) when the cells were treated with 1, 10, 100, and 1,000 nmol/L fulvestrant, respectively.

To assess whether changes in 18F-FES uptake could be attributed to competitive binding with fulvestrant or changes in total receptor concentration, we determined ERα protein expression in cells treated in parallel. Dose-dependent changes in ERα protein levels were observed (Fig. 2B). The $IC_{50}$ was 4.4 nmol/L (95% CI, 3.0 × 10^{-10} to 6.4 × 10^{-8} mol/L; model $R^2$: 0.613), a potency similar to that observed by 18F-FES uptake. However, the ERα expression downregulation in MCF7 cells paralleled the reduction seen with 18F-FES uptake with fulvestrant treatment.

To account for upstream changes in gene expression, we measured the level of ESR1 mRNA transcripts following treatment. Fulvestrant induced a dose-dependent increase in ESR1 expression (Fig. 2C; ~3-fold), possibly reflecting negative feedback loop relief. The 50% effective concentration was 0.17 nmol/L (95% CI, 1.8 × 10^{-11} to 1.6 × 10^{-8} mol/L; model $R^2$: 0.596). These data suggest that, in breast cancer cells in vitro, fulvestrant downregulates ERα specifically at the protein level, a dose-dependent change that is captured by 18F-FES uptake.

**Ex vivo analysis of tumor xenografts**

In MCF7 xenografts resected 48 hours after fulvestrant treatment, nuclear ERα protein expression displayed significant dose-dependent changes with fulvestrant treatment (Fig. 3A; $P = 0.001$). Compared with vehicle, tumor ERα protein levels decreased by 30.6% ($P = 0.03$), 73.9% ($P = 0.0015$), and

![Figure 3](image-url)

**Figure 3.**

Ex vivo analyses of MCF7 xenografts. Nude mice bearing MCF7 xenografts were given one dose of fulvestrant 48 hours before tumors were extracted for analysis ($n = 2–3$ mice per group). A, ERα expression versus fulvestrant dose as determined by sandwich ELISA. Means were compared with vehicle control. There was a significant reduction in ERα expression level with fulvestrant treatment (ANOVA; $P = 0.001$). The reduction in ERα expression was more pronounced with higher doses of fulvestrant. B, ESR1 mRNA expression versus fulvestrant dose as determined by qRT-PCR. There was no significant difference among treatment groups in ERα mRNA expression. $^*$, $P < 0.05$; $^{**}$, $P < 0.01$. Error bars are SEM.

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Clin Cancer Res; 21(6) March 15, 2015

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Published OnlineFirst January 21, 2015; DOI: 10.1158/1078-0432.CCR-14-1178

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74.9% (P = 0.0015) when mice were treated with 0.05, 0.5, and 5 mg fulvestrant, respectively. These changes in protein expression from tumor lysate were confirmed by ER IHC. Qualitative analysis revealed reduced nuclear ER staining intensity and proportion of positive cells in tumors from mice treated with higher fulvestrant doses (Fig. 4A–D).

Upstream of ER protein expression, dose-dependent changes in ESR1 mRNA expression were not apparent (Fig. 3B; P = 0.39). Similarly, treatment was not associated with discernible reductions in staining for the proliferation marker Ki67 (Fig. 4E–H). Most cells in non-necrotic areas of all tumors remained highly positive for Ki67, indicating little effect on downstream ER function. Together, these data confirm that fulvestrant degrades ER specifically at the protein level in a dose-dependent manner in vivo and that these changes occur before changes in proliferation.

**In vivo PET imaging**

We assessed whether quantitative molecular imaging with 18F-FES demonstrates the dynamic range and sensitivity necessary to reveal early changes in fulvestrant-induced ER degradation/blockade at pharmacologic doses. Mean 18F-FES PET SUVmean in MCF7 xenografts was significantly different among groups 48 hours after fulvestrant treatment (Fig. 5A; P = 0.0001). Tumor SUVmean was 0.33 ± 0.02 for vehicle-treated mice and 0.33 ± 0.04 for low-dose (0.05 mg) fulvestrant-treated mice, which were not significantly different (P = 0.86). However, compared with vehicle treatment (control), fulvestrant treatment resulted in reduction of SUVmean to 0.19 ± 0.03 (P = 0.0054) at 0.5 mg and to 0.14 ± 0.02 (P = 0.0006) at 5 mg doses. Compared with low-dose fulvestrant (0.05 mg), both 0.5- and 5-mg fulvestrant doses significantly reduced SUVmean (P = 0.009 and P = 0.0011, respectively). There was no significant difference in 18F-FES uptake between 0.5- and 5-mg treatment groups (P = 0.33).

The TBR of MCF7 xenografts in 18F-FES PET imaging were 4.17 ± 0.41, 4.00 ± 0.62, 2.24 ± 0.30, and 1.49 ± 0.33 in the vehicle, 0.05-, 0.5-, and 5-mg treatment groups, respectively. The difference in the mean TBR among different groups was significant (P = 0.0005). The mean TBR was significantly lower in 0.5- and 5-mg dose groups than the vehicle control group (P = 0.013 and P = 0.0017, respectively). There was no significant difference in the mean TBR of 0.05 mg and vehicle-treated groups (P = 0.79), nor was there any difference in the mean TBR of 0.5 mg and 5-mg treatment groups (P = 0.4).

Downstream metabolic changes were not evident 3 days after fulvestrant treatment. Mean SUVmean of MCF7 xenografts in 18F-FDG PET in the vehicle, 0.05 mg, 0.5 mg, and 5 mg treatment groups were 1.40 ± 0.11, 1.50 ± 0.17, 1.47 ± 0.18, and 1.41 ± 0.09, respectively. There was no significant difference in SUVmean among any of the dose groups in 18F-FDG PET imaging (Fig. 5C; P = 0.94). Representative images for all fulvestrant doses for both 18F-FDG and 18F-FES PET/CT are shown in Fig. 6.

There was no correlation between 18F-FDG and 18F-FES SUVmean in MCF7 xenografts (Fig. 5D; r = −0.038, R² = 0.0014, P = 0.89). The lack of correlation between 18F-FES and 18F-FDG PET imaging results is concordant with the histopathologic analysis of xenograft samples in different treatment groups. The discrepancy between the 2 imaging sets suggests that changes in ERα availability occur much earlier than downstream effects on tumor metabolism and proliferation. Collectively, these data suggest that 18F-FES-PET is able to interrogate fulvestrant-induced pharmacodynamic changes before other in vitro or in vivo biomarkers of treatment effectiveness.

**Discussion**

Fulvestrant is approved by the FDA for treatment of ER-positive metastatic breast cancer in postmenopausal women with disease progression while on antiestrogen therapy. Despite the proven efficacy of fulvestrant, the optimal biologic dose of this drug in breast cancer treatment has been an area for debate. It is hypothesized that the lower-than-expected clinical performance of fulvestrant in breast cancer may be explained by inadequate fulvestrant levels at standard recommended dosing levels, supported by recent reports of increased efficacy at higher doses (17, 32).

Pooled analysis of clinical trials demonstrated an actual dose-dependent clinical response. The CONFIRM trial demonstrated that high-dose fulvestrant (500 mg/mo) produces a statistically significant and clinically relevant prolongation of progression-free survival (PFS) and duration of clinical benefit over a low-dose regimen (250 mg/mo), whereas the adverse effects profile remains essentially comparable with both dosing schemes (17, 18).

18F-FES, an ER-specific radiotracer, has previously been employed for quantitative evaluation of ER expression in breast cancer in clinical trials (21, 27, 29, 33, 34). Our findings support the ability of 18F-FES PET to visualize the in vivo activity of...
endocrine therapy. In both in vitro and in vivo experiments, we demonstrated that 18F-FES uptake in breast cancer cells decreases in a dose-dependent manner in response to fulvestrant treatment. Our results are concordant with early findings in a retrospective study by Linden and colleagues that showed the incomplete tumor blockade of 18F-FES uptake with fulvestrant may indicate a potential explanation for the lower-than-expected clinical performance of fulvestrant (29). These findings have been recently confirmed in a prospective study, which demonstrated early progression of metastatic breast cancer during the first 3 months of fulvestrant therapy was related to significant residual 18F-FES uptake (34). These findings match our findings and indicate that 18F-FES may be used as a biomarker for fulvestrant efficacy and may help identify patients that could potentially benefit from a higher dose.

To the best of our knowledge, we have demonstrated for the first time that a graded response to fulvestrant can be monitored by 18F-FES and the magnitude of reduction in radiotracer uptake was concordant with changes in functional ER expression. The reduction in 18F-FES uptake by xenografts could be largely explained by reduced ER expression due to protein ubiquitination and degradation and/or direct blockade of ER by fulvestrant. We observed a parallel but slightly greater reduction in 18F-FES uptake compared with ER expression level changes with fulvestrant treatment. This suggests that the reduction in 18F-FES uptake is mainly due to diminished ER expression, whereas direct blockade of ER by fulvestrant contributes to a considerably smaller part of the imaged change at the time point used in our study. Cancer cell apoptosis is a distant third possible minor cause for reduction in 18F-FES uptake, given there was no change in proliferation, tumor size, or 18F-FDG uptake at the time of 18F-FES PET imaging. In this regard, it is important to note that the alterations in 18F-FES uptake by SERDs should be cautiously attributed to changes in the ER expression. To further delineate the etiology of these effects, repeat tissue biopsy and/or multi-time point 18F-FES PET imaging would provide additional insight.

We demonstrated 18F-FES uptake highly correlated with ER protein levels but not mRNA expression. It has been shown that ESR1 mRNA robustly correlates with protein expression and holds prognostic significance (35, 36). However, because SERD therapy uncouples ER protein expression from ESR1 mRNA transcription by specifically degrading ER protein, 18F-FES makes a better prognostic tool than ESR1 mRNA for functional ER levels in the setting of such targeted therapy.

In this study, we observed dissociation between dose-dependent reduction in ER expression and 18F-FES uptake and the lack of changes in tumor cell proliferation (Ki67 staining) and metabolism (18F-FDG uptake), which were not yet apparent after fulvestrant treatment, at least up to 48 hours posttreatment (the time point evaluated in the current study). This is in contrast to the results from clinical studies indicating that ER and the cell proliferation marker, Ki67, were downregulated with fulvestrant treatment (13, 14, 37). We used similar doses of fulvestrant given in widespread reports in similar preclinical tumor model (31, 38, 39). However, in our study, the time between treatment and tumor extraction was much shorter than previous reports and was likely insufficient for ER degradation to affect subsequent downstream metabolic and proliferation pathways. This represents a unique advantage for 18F-FES in identifying early pharmacodynamic changes and demonstrates how imaging steroid hormone receptors can be used for dose optimization of fulvestrant based on functional, unoccupied ER measurements. Thus, 18F-FES could be a better surrogate for optimized fulvestrant dosing in individuals with breast cancer compared with other
noninvasive assessments such as tumoral 18F-FDG uptake. Such personalized treatment may provide additional benefit over standard uniform dosing approaches currently employed and allows for early alternative intervention for nonresponsive tumors.

There are several limitations to this study. First, endocrine therapies that directly block ER (e.g., fulvestrant, tamoxifen, and raloxifene) or diminish ER protein levels (e.g., fulvestrant) both can decrease the 18F-FES uptake by tumor. Thus, it is not possible to determine what proportion of change in 18F-FES uptake is due to either effect. Second, measuring receptor occupancy levels using 18F-FES PET is most useful when the receptor-targeted therapies such as tamoxifen or fulvestrant are in the subsaturating range. If the administered dose of anti-estrogen is high enough to completely saturate receptors, then there will be no receptor-specific tracer uptake; in these circumstances, receptor quantitation using PET imaging shows complete occupancy and the degree of excess drug dose cannot be assessed. In practice, the majority of patients with breast cancer is given subsaturating doses of anti-ER therapy and may benefit from dose adjustment using the proposed approach. Third, the data presented in this study are derived from experiments on a well-established model of ER+ breast cancer that is commonly used. Using this model, we demonstrated the feasibility of pharmacodynamic imaging for improved fulvestrant dosing. However, as the model we used was only derived from MCF7 cells, generalizing these findings may require further studies with multiple breast cancer models.

This study provides preliminary evidence that responsiveness to endocrine therapy could be measured early in the course of ER-targeting therapies by quantitative imaging of ER binding in breast cancer. In this regard, serial 18F-FES PET imaging provides a quantitative assessment of changes in receptor binding caused by therapeutics aimed to induce receptor blockade/degradation and thus has the potential to refine treatment selection and dosing in individual patients. In particular, 18F-FES PET could notify the oncologist early in the course of treatment of inadequate anti-ER therapy and guide improved dosing of anti-ER therapeutics. This technology could also be used early in drug development to measure effectiveness at the intended therapeutic targets and to help refine patient selection and dosing levels for agents in drug development. In addition, this image-guided approach to individualized drug dosing may also be employed for other receptor-targeted therapies in cancer treatment in the future. Given the limitations of this study, further study using carefully timed serial 18F-FES PET imaging is warranted to assess the role for this imaging tool in ER-targeted therapies in the clinical setting.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): P. Heidari, F. Deng, S.A. Esfahani, A.K. Leece
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P. Heidari, F. Deng, S.A. Esfahani, A.K. Leece, U. Mahmood
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Acknowledgments

The authors thank Anna Levitz, MGH Specialized Histopathology Services, and Caitlin Routhier, MGH Immunopathology Unit, for technical assistance with IHC. They also thank Bryan Chang for helpful discussions and technical assistance with maintaining tumor-bearing mice and Nazife Selcan Turker for technical assistance with radiotracer administration.

Grant Support

This work was supported by U01CA084301 and P50CA127003. F. Deng supported by a Bradley-Alavi Student Fellowship from the Society for Nuclear Medicine and Molecular Imaging and a Washington University School of Medicine Dean’s Fellowship.

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Received May 8, 2014; revised October 12, 2014; accepted October 13, 2014; published OnlineFirst January 20, 2015.

References


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