Imaging, Diagnosis, Prognosis

Clinical Cancer Research

Fluoroestradiol Positron Emission Tomography Reveals Differences in Pharmacodynamics of Aromatase Inhibitors, Tamoxifen, and Fulvestrant in Patients with Metastatic Breast Cancer

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Abstract

Purpose: To determine, by molecular imaging, how *in vivo* pharmacodynamics of estrogen-estrogen receptor (ER) binding differ between types of standard endocrine therapy.

Experimental Design: The ER has been a highly successful target for breast cancer treatment. ER-directed treatments include lowering ligand concentration by using aromatase inhibitors (AI) and blocking the receptor with agents like tamoxifen (TAM) or fulvestrant (FUL). We measured regional estrogen-ER binding by using positron emission tomography with ^{18}F-fluoroestradiol (FES PET) prior to and during treatment with AI, TAM, or FUL in a series of 30 metastatic breast cancer patients. FES PET measured *in vivo* estrogen binding at all tumor sites in heavily pretreated women with metastatic bone soft tissue–dominant breast cancer. In patients with uterus ($n = 16$) changes in uterine FES uptake were also measured.

Results: As expected, tumor FES uptake declined more markedly on ER blockers (TAM and FUL, average 54% decline) compared with a less than 15% average decline on estrogen-depleting AIs ($P < 0.001$). The rate of complete tumor blockade [FES standardized uptake value (SUV) $\leq$1.5] following TAM (5/5 patients) was greater than the blockade rate following FUL (4/11; 2-sided mid $P = 0.019$). Percent FES SUV change in the uterus showed a strong association with tumoral change ($\rho = 0.63$, $P = 0.01$).

Conclusions: FES PET can assess the *in vivo* pharmacodynamics of ER-targeted agents and may give insight into the activity of established therapeutic agents. Imaging revealed significant differences between agents, including differences in the efficacy of blockade by different ER antagonists in current clinical use. *Clin Cancer Res; 17(14); 4799–805. ©2011 AACR.*

Introduction

Targeting the estrogen receptor (ER) is an established treatment strategy for hormone-sensitive breast cancer. Two common and effective breast cancer treatments are ER blocking agents targeting the receptor, and estrogen depleting agents targeting production of the ligand. ER blockers include selective ER modulators such as tamoxifen (TAM), which have both ER agonist and antagonist properties, working primarily as antagonists in tumors. A second class of ER blockers includes fulvestrant (FUL), which is a selective ER downregulator (SERD) and a pure ER antagonist. The most successful ligand-depleting agents to date have been aromatase inhibitors (AI; ref. 1) Estrogen-lowering AIs target aromatase, the enzyme that converts androstenedione and testosterone to estrogen, and result in lower estrogen levels both in the plasma and at the tumor site (2–4). Despite the success of ER-directed therapy, important clinical dilemmas remain. Only 50%–75% of ER-expressing breast cancer tumors benefit from hormonal therapy as first line therapy (4, 5), and fewer patients respond to alternative endocrine agents once they have failed another endocrine treatment (5, 6). Preclinical models have studied the effect of endocrine agents on the ER (7–10), suggesting that ER expression may be downregulated with long-term exposure. However, our ability to measure pharmacodynamics of endocrine therapy in breast cancer patients has been limited, and mechanisms of resistance are incompletely understood.

One tool that holds promise for measuring drug effects in cancer patients is molecular imaging (11). We and others have previously shown that functional ER imaging by using 16α-[^{18}F]-fluoroestradiol positron emission tomography (FES PET) measures regional ER binding (12, 13) and identifies which patients have tumors likely to show a response to endocrine therapy (14, 15). Other studies have shown that serial FES can show ER blockade in patients

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Translational Relevance

The estrogen receptor (ER) is a critical target in breast cancer. Pharmacodynamic imaging, by using an estradiol tracer, has the ability to evaluate *in vivo* ER concentration of estrogen. Serial imaging provides a quantitative assessment of changes in receptor binding induced by proven therapeutics aimed to induce receptor blockade or ligand depletion. Receptor blocking therapies induce significantly greater reduction in ER uptake than ligand depleting agents, aromatase inhibitors (AI). Receptor blockade with tamoxifen [a selective ER modulator (SERM)] was more pronounced than fulvestrant [a selective ER downmodulator (SERD)]. These findings are consistent with the clinical outcomes observed with endocrine therapies, and show the impact of AIs, SERMs, and SERDs on tumor and normal tissue. Serial FES has the potential to refine treatment selection and provide *in vivo* pharmacokinetic evaluation to guide drug development.

treated with TAM (14, 16, 17). On the basis of these findings, we examined the ability of FES PET to measure *in vivo* pharmacodynamic effects of several currently used endocrine agents to yield insights into their clinical efficacy and to suggest potential mechanisms of resistance. FES PET is a functional assay that measures the tumor's ability to bind estradiol, as indicated by trapping of FES. Complementary to *in vitro* assay of ER expression, FES PET measures *in vivo* ER function and can assess the whole-body tumor burden. Quantitative assessment of ER binding in imaging studies has shown that an average FES PET standardized uptake value (SUV) more than 1.5 is associated with response (partial or complete) to ER-targeted therapy (14, 15), and importantly, SUV of 1.5 or less predicted a lack of response, suggesting that an SUV of 1.5 is a threshold for predicting responsiveness to endocrine therapy.

To measure the effect of endocrine therapy on regional estradiol binding to ER in breast cancer lesions, we measured tumor FES uptake prior to, and during endocrine therapy. We also measured changes in uterine FES uptake, a normal organ with high ER expression, to test the extent to which changes in uterine uptake match changes in tumor FES uptake, under the hypothesis that the uterus might serve as an indicator of the effect of endocrine therapy on estradiol binding in tumors. Our underlying hypothesis was that ER antagonists such as TAM and FUL would cause a decline in tumor and uterine FES uptake, whereas AIs would have little impact on FES uptake. In addition, we also sought to investigate differences between ER antagonism for different blocking agents used in the clinic, namely TAM and FUL.

Materials and Methods

Patients

For this retrospective analysis, we identified patients with metastatic breast cancer who underwent serial FES imaging under endocrine-directed therapy. From 1996 to 2006, 391 FES scans were carried out at the University of Washington Medical Center under a variety of research protocols. Among the 312 scans of 239 patients with ER-positive primary disease and visible tumor (see Peterson and colleagues; ref. 18 for further details), 51 had multiple scans and 30 of these met the following study entry criteria. Patients selected for analysis had metastatic (primarily bone dominant) breast cancer and were undergoing salvage endocrine therapy. Concomitant cytotoxic therapy resulted in exclusion from this analysis, but concomitant trastuzumab and bisphosphonates did not.

Endocrine therapy selection and dosage were determined clinically by the treating physician, following standard clinical practice [20 mg per os (po) daily for TAM, 1 mg po daily for anastrozole, 2.5 mg po daily for letrozole, and 25 mg po daily for exemestane]. FUL was administered in most (8/11) patients as per an ongoing clinical protocol at a loading dose of 500 mg, X1, followed by 250 mg at 2 weeks X2. Two patients were given a second 500 mg loading dose, and 1 was given only 250 mg monthly without a loading schedule or increased dose. The majority of patients who started FUL after the baseline FES PET (10/11) were already on chronic AI therapy at the time of initial PET and had experienced disease progression.

FES PET imaging and image analysis

FES PET was carried out pretherapy and at 1 to 18 weeks after starting therapy (median of 6 weeks). All patients were required to be off ER blocking agents (TAM and FUL) for a minimum of 60 days prior to the pretherapy (baseline) FES PET scan. All patients provided informed consent and study protocols were approved by the University of Washington's Institutional Review Board (Seattle, WA) and Radioactive Drug Research Committee.

Synthesis of FES was carried out as previously described (15). Specific activity was measured for each administration, and in no case was more than 5 mcg of FES injected. FES was infused through an intravenous catheter over 2 minutes in a volume of 20 mL of saline. Dynamic imaging was carried out over an imaging field containing 1 or more of the largest sites of disease from injection to 60 minutes, as previously described (15). Dynamic imaging was followed by a torso survey from skull base to thighs consisting of a 5-minute emission image and 3-minute transmission imaging per 15 cm axial imaging field. Images were corrected for scattered and random coincidences and reconstructed by using an ordered subset expectation maximization algorithm for qualitative image review and filtered back-projection by using a Hanning filter for quantitative analysis.

In image analysis, active sites of disease were identified by correlation to standard imaging [computed tomography (CT); bone scan] and PET with 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG) taken close to the time of FES PET. FDG PET was used only to identify active sites of disease to guide placement of regions-of-interest (ROI) for the FES scans (15). The position of the uterus was identified by

reference to pelvic CT coregistered to the FES PET images. Coregistration and ROI placement was done by using PMOD software (PMOD Technologies Ltd). Images were qualitatively reviewed for the presence or absence of uptake at known sites of active disease by a single highly experienced observer. Quantitative FES tumor uptake was measured as the average FES SUV for up to 3 of the most prominent tumor sites imaged (15). In patients with intact uteri, uterine FES uptake was also evaluated. In quantitative measurements of regional ER binding, a threshold of FES SUV 1.5 was used as an indicator of endocrine responsiveness, as previously identified in studies of the predictive value of FES uptake (15). Limited data (repeat imaging in a single patient within 7 days without intervening therapy) from our center with FES, and for FDG PET from our center and others (19) suggest repeatability. The goal of this study was to examine the pharmacodynamic response to different classes of endocrine therapy (estrogen depleting agents vs. blocking agents). As such, tumor and uterine FES SUV at follow-up, and percent change in FES SUV, were the primary study end points.

Statistical methods

Percent change in FES SUV was compared for blocking and nonblocking agents by using a 2-sample *t* test after checking normality assumptions by visual inspection. The rate of blockade (defined as noted above as FES SUV $\leq$1.5 at follow-up) was assessed for TAM and FUL by using the mid *P* value correction to Fisher's exact test (20, 21). Paired tumor and uterus data were compared by using Spearman's rank correlation. Because trastuzumab may restore ER levels after extended AI therapy (22), a sensitivity analysis excluded patients with concomitant trastuzumab. Statistical analyses were conducted by using R version 2.8.1 (R Foundation for Statistical Computing).

Results

Patient characteristics

We conducted serial FES PET imaging in 30 patients (Table 1) undergoing endocrine therapy for metastatic breast cancer, to determine if regional ER binding was modulated by hormonal therapy. Sixteen of these

Table 1. Patient characteristics and imaging results

	Tumor (n = 30)		Tumor AI (n = 14)		Tumor FUL (n = 11)		Tumor TAM (n = 5)		Uterus subset (n = 16)	
	Median	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**
Patient age, y	55	28–77	49	35–70	59	28–77	55	45–71	48	28–77
Time between scans (wk)	6	1–18	4	1–14	9	4–18	5	3–7	4.1	3–17
Baseline FES SUV	2.9	0.9–6.5	2.3	1.7–3.9	3.8	0.9–6.5	2.3	1.2–3.2	5.2	1.6–7.7
Follow-up FES SUV	1.8	0.4–4.1	2.0	1.0–4.1	2.1	0.4–3.6	0.7	0.5–1.2	3.5	0.9–8.1
(%) change FES SUV	−42	−84–38	−13	−56–38	−49	−84–9	−55	−77–46	−16	−84–51
	n	**%**	***N***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
Patient sex										
Female	27	90	14	100	10	91	3	60	16	100
Male	3	10	0	0	1	9	2	40	0	0
Tumor histology										
Ductal	27	90	12	86	11	100	4	80	15	94
Lobular	3	10	2	14	0	0	1	20	1	6
HER2/neu										
Positive	7	23	4	29	2	18	1	20	5	31
Negative	23	77	10	71	9	82	4	80	11	69
Concomitant trastuzumab										
Yes	5	17	3	21	2	18	0	0	4	25
No	25	83	11	79	9	82	5	100	12	75
Treatment										
AI	14	47								
ER blocker	16	53								
FUL	11									
TAM	5									

patients had an intact uterus, which served as a secondary site for analysis of ER binding. The majority of patients were postmenopausal women with bone soft tissue–dominant metastatic breast cancer. Nearly half were on AI therapy, and just over half were on ER blocking therapy (11 FUL, 5 TAM). Seven patients were $HER2^{+}$, and 5 (3 on AI and 2 on FUL) were treated with trastuzumab while on salvage endocrine therapy. Two were continuing trastuzumab, given with prior regimen(s), and 3 initiated trastuzumab with salvage endocrine therapy given following baseline FES.

Image examples

A representative TAM patient shown in Figure 1A had recurrent bone metastasis at the thoracic spine and an ER-positive primary lesion. In the 21 days between FES scans, the spinal tumor average SUV decreased from 2.7 to 0.6, and SUV of the uterus decreased from 6.9 to 1.1. An image set of a patient undergoing FUL treatment with bony metastasis is shown in Figure 1B. The posttreatment image is 68 days after a loading dose of FUL. The change in spinal tumor SUV was from 6.1 to 3.6 and uterine SUV from 4.3 to 0.9. Figure 1C shows images for a patient on an AI, letrozole, for 29 days between pre- and posttreatment imaging. In this case, average tumor SUV decreased from 2.1 to 1.8 and the uterus SUV from 7.1 to 4.7.

Changes in tumor FES uptake with therapy

Baseline average FES SUV ranged from 0.9 to 6.5 (Table 1, Fig. 2A). There was a trend for baseline SUV to be greater for patients receiving blocking therapy (mean 3.4) versus nonblockers (mean 2.6; $P = 0.11$). Figure 2A shows that this difference was driven by the patients taking FUL. In contrast (Table 1, Fig. 2C), the average FES SUV for patients during blocking therapy was lower (mean 1.5) compared

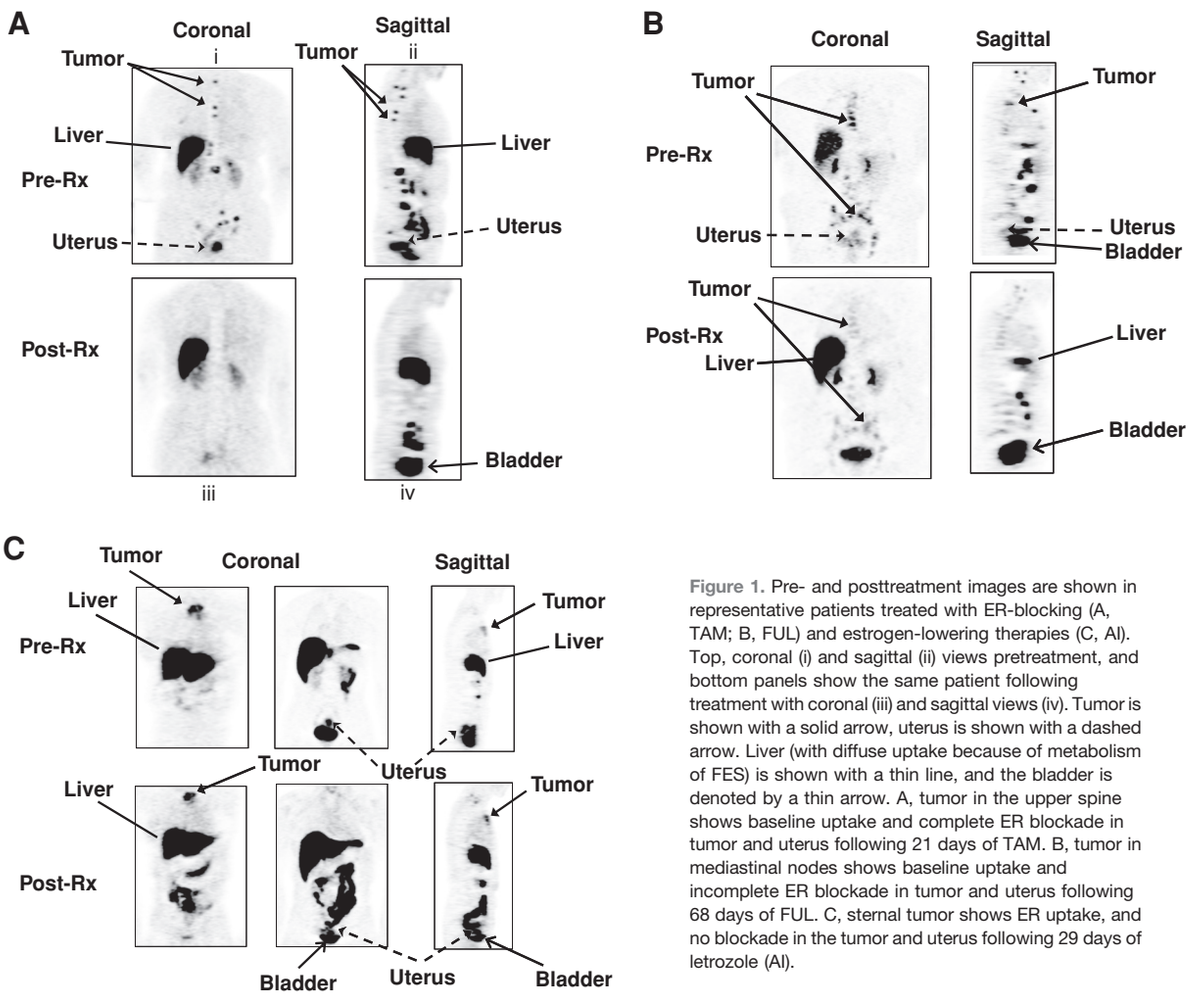

Figure 1. Pre- and posttreatment images are shown in representative patients treated with ER-blocking (A, TAM; B, FUL) and estrogen-lowering therapies (C, AI). Top, coronal (i) and sagittal (ii) views pretreatment, and bottom panels show the same patient following treatment with coronal (iii) and sagittal views (iv). Tumor is shown with a solid arrow, uterus is shown with a dashed arrow. Liver (with diffuse uptake because of metabolism of FES) is shown with a thin line, and the bladder is denoted by a thin arrow. A, tumor in the upper spine shows baseline uptake and complete ER blockade in tumor and uterus following 21 days of TAM. B, tumor in mediastinal nodes shows baseline uptake and incomplete ER blockade in tumor and uterus following 68 days of FUL. C, sternal tumor shows ER uptake, and no blockade in the tumor and uterus following 29 days of letrozole (AI).

with patients on nonblockers (mean 2.2; $P = 0.04$). The average percent decline in FES SUV for patients on blocking agents was 54% whereas the average change for patients on AIs was 14%. As shown in Table 1 and Figure 2E, the average percent decline in FES SUV for patients on blocking agents was greater than the average change for patients taking AIs ($P < 0.001$).

Follow-up scans (Fig. 2C) show that all patients on TAM (5/5), but only a minority of patients treated with FUL (4/11, 36%), showed complete blockade of tumor FES uptake (SUV $\leq$1.5) posttherapy. For patients undergoing AI therapy, 3/14 (21%) had posttherapy FES SUV of 1.5 or less. The overall 56% rate of posttherapy SUV or 1.5 or less for blocking therapies (TAM and FUL) was greater than the rate for AI therapy (2-sided mid $P = 0.045$). However, TAM appeared to be a more effective blocker of tumor ER binding than FUL (5/5 vs. 4/11; 2-sided mid $P = 0.019$). All these results were essentially unchanged by removing the 5 patients treated with trastuzumab. Four of these patients are identified by red plotting characters in Figure 1; the fifth is the FUL patient with a blue plotting character (for low baseline FES uptake).

Two of 30 patients (7%) had baseline tumor uptake below the threshold for endocrine responsiveness (SUVs 1.18 and.87). The effect of blocking therapy in patients with low ER binding was tracked in Figure 2C and E as solid blue plotting characters for these 2 patients (1 taking FUL and 1 TAM). Both patients showed an FES SUV decrease of about 50%, similar to other patients on blocking therapies.

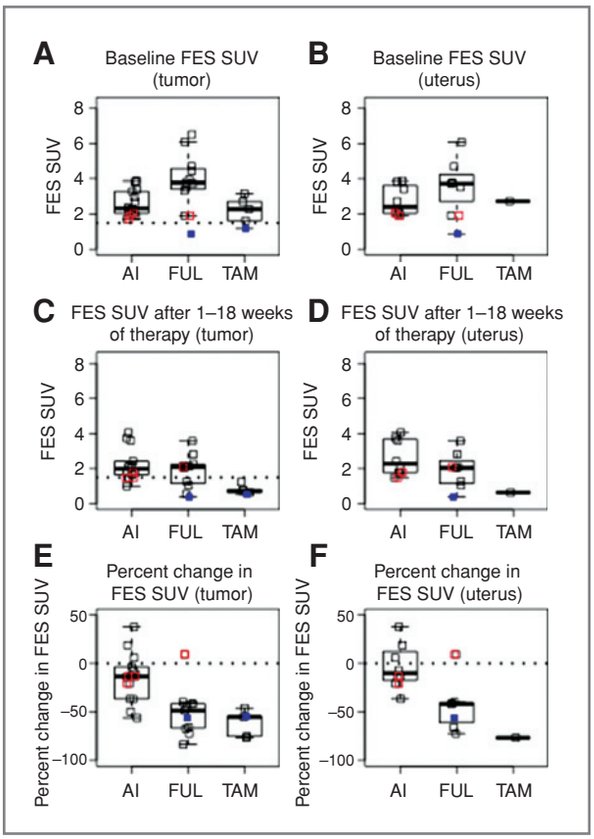

Figure 2. Quantitative tumor and uterine FES SUV in patients undergoing AI, FUL, or TAM treatment are shown with box and whisker plots superimposed. FES SUV values are shown at baseline [tumor site(s), A; uterus, B], and after 1 to 18 weeks of therapy [tumor site(s), C; uterus, D). Percent change between baseline and follow-up is shown for tumor site(s) (E) and the uterus (F). Two patients had baseline tumor uptake below the threshold for blockade (FES SUV $\leq$1.5) and are denoted by solid blue squares to track the effect of blocking therapy on tumors with low uptake. The FUL patient with low uptake and 4 additional patients (indicated by red plotting characters) took concomitant trastuzumab during endocrine therapy. Horizontal dashed lines indicate the FES SUV $\leq$1.5 threshold for tumor blockade (A and C) and a 0% change threshold for percent change in SUV (E and F).

Timing of FES and change in uptake

To evaluate the potential for the timing of the second FES scan to confound our analysis, we analyzed the impact of the timing of the second FES on the follow-up FES SUV and on the percent change in uptake, for each type of treatment (see Supplementary Fig. S1A and B). In linear regression models holding the treatment group constant, there was no association between time intervals and follow-up FES SUV (−0.004 average difference per day $P = 0.50$) or percent change (−0.04% average difference per day; $P = 0.82$).

Uterine uptake and comparison with tumor uptake

At the uterus ($n = 16$; Fig. 2F), the average change in FES SUV was a 51% decrease after blocking therapy (46% mean decrease after FUL treatment and 84% mean decrease after TAM treatment), compared with a 4% increase after AI treatment ($P < 0.001$). As for the tumor sites, the rate of low FES uptake posttherapy was greater for patients undergoing blocking therapy [3/8 (38%) with uterine FES SUV $\leq$1.5] than for patients undergoing AI therapy (0/8, 2-sided mid $P = 0.10$). Uterine blocking of ER binding under FUL treatment appeared incomplete in most patients (Fig. 2D). The only patient undergoing TAM therapy with an intact uterus showed complete uterine blockade of FES uptake.

The right side of Figure 2 and these quantitative results suggested that functional imaging of the uterus largely mirrors the patterns seen in tumor sites undergoing endocrine therapy. We next examined the paired tumor-uterus FES data. Spearman rank-order correlation did not find an association between baseline uterine and tumor uptake ($\rho = 0.11$; $P = 0.68$) or follow-up uterine and tumor uptake ($\rho = 0.39$; $P = 0.14$). However, there was a strong association between percent changes in FES SUV in the tumor and the uterus ($\rho = 0.63$; $P = 0.01$).

Discussion

FES PET estimates regional binding of estrogens to the ER, which suggests the use of FES PET imaging to assess the phamacodynamic effect of ER-directed drugs on estrogen-ER binding. Our observational data showed that estrogen-depleting therapies did not impact the tumor's

ability to bind FES, whereas, as expected, blocking therapies mitigated FES uptake *in vivo*. We found that estrogen blocking therapies (TAM and FUL) were effective at decreasing FES binding at the tumor and uterus, in contrast with AIs which did not generally affect estrogen binding. This difference between blocking and nonblocking therapies was expected but shows the ability of molecular imaging to measure drug effects *in vivo* at multiple tumor sites in patients undergoing endocrine therapy. Interestingly, imaging showed unexpected differences in blockade between 2 commonly used ER antagonists, TAM and FUL. This has not been previously reported and was not seen in preclinical studies (23). The incomplete tumor blockade seen with FUL may explain its lower than expected clinical performance in humans (24). This may be the result of inadequate FUL levels at standard recommended dosing levels, supported by recent reports of increased efficacy at higher doses (25, 26).

Although a slight increase in FES SUV postligand depletion was expected as a result of decreased competition between local estrogens and FES, there was a trend for a greater decline in FES SUV with time on AI between scans. This interesting finding could be the result of cellular dropout and decline in tumor cells per unit volume or to a decline in receptor expression in the cancer cells. Considering preliminary studies that showed decline in FDG uptake after AIs (27), we suspect cellular dropout as the mechanism for this change; however, determination of the precise mechanism underlying this finding will require further study. Clarification of the mechanism underlying the decline in FES uptake with AI therapy could be obtained from additional studies with serial biopsy or serial imaging by using closely timed FES and FDG or [^{18}F]-fluorthymidine PET to compare tumor cellularity, proliferation, and *in vitro* ER expression pre- and post-AI treatment.

One goal of our study was to examine the extent to which the uterus could serve as an indicator of the effect of endocrine therapy on estradiol binding in tumor ER. We found, in general, relatively similar changes in FES uptake for tumor and normal uterus for the subset of study patients with an intact uterus. This suggests that the uterus may serve as a proxy for tumor ER binding in some studies. This could be helpful in studies of drug metabolism or interfering agents, where ethically we would not want to study effects which might limit endocrine therapy efficacy in patients with known breast cancer.

There are several limitations to this study. An important limitation is that the interval between baseline and follow-up scans was not uniform, in part because of different loading schedules for each treatment regimen. Although we found some trend for changes in posttherapy FES uptake for AIs (see above and Supplementary Figure), there was no similar trend for the blockers. A second limitation, understandable for early experimental imaging studies, was a small number of patients in each category; nevertheless, we find significant differences between AIs and ER-blocking drugs and between the degree of blockade for TAM versus FUL. However, results will need further validation in larger controlled studies.

Another limitation of this study is that imaging was carried out at the time of switch in endocrine therapy as clinically indicated with the many patients already undergoing AI treatment (experiencing response initially, followed by progression) to which FUL was added. Although we would not expect an interaction between AI treatment and the FUL blockade of the receptor, such an interaction cannot be excluded on the basis of our study. An additional limitation of our study is that concomitant trastuzumab could alter ER expression (22); the limited number of patients who received trastuzumab, and the fact that trastuzumab was continued in some of these patients, not added at the time of a switch in therapy, reduces the chance of pathway crosstalk. In addition, patients were undergoing salvage endocrine therapy after a variety of prior therapies. The FES PET method is limited because it measures binding only, not downstream function or other growth-promoting pathways; however, its sensitivity for measuring ER expression is similar to immunohistochemistry (12). As noted, imaging cannot separate changes in uptake resulting from reduced-ER expression or binding per tumor cell from changes related to tumor cellularity. However, companion FDG PET imaging may provide an indirect measure of response to treatment and change in tumor cellularity (28).

Conclusions

Our findings support the ability of FES PET to visualize the *in vivo* activity of endocrine therapy. This technology could be used early in drug development to measure effectiveness at the intended therapeutic targets, to help refine selection and dosing for agents to move forward in drug development. In addition, pharmacodynamic imaging could provide clinicians with a promising tool for therapeutic selection and for predicting and evaluating response to ER-targeted therapy. Whole-body PET imaging has the advantage of monitoring ER binding at multiple sites. This study provided preliminary evidence that responsiveness to endocrine therapy could be measured by serial studies of ER binding in the uterus for patients whose primary tumors are small and/or resected. Given these findings, further study using carefully timed serial FES imaging is indicated to determine the role for this imaging tool in ER-targeted drug development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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