ELSEVIER

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^{18}F-Fluoroestradiol

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Estrogen receptor (ER) expression is an important determinant of breast cancer behavior and is critical for response to endocrine therapies such as tamoxifen and aromatase inhibitors. In current practice, ER expression is determined by assay of biopsy material. In more advanced disease, tissue assay may present practical difficulties and be associated with significant sampling error. This and other considerations motivated the development of ER imaging agents for positron emission tomography (PET), of which the most successful has been ^{18}F-16α-17β-fluoroestradiol (FES). In this review, we highlight aspects of ER biology and the importance of the ER in breast cancer therapy; review the structure and synthesis of FES; describe its kinetics and safety/dosimetry data; and highlight validation studies. Also discussed are early results in patients using FES-PET to localize ER-expressing tumors and associated data pointing toward its accuracy as a predictive assay for breast cancer endocrine therapy. Finally, early data for tumors and sites other than breast cancer are mentioned. Preliminary data strongly point toward potential clinical utility for FES-PET, motivating further validation and future clinical trials with prospective endpoints tested under appropriate regulatory oversight.
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Approximately 83,000 women present with advanced breast cancer each year in United States, and most have estrogen receptor-expressing (ER+) tumors.[1] The ER is a target for breast cancer treatment using endocrine therapy. Strategies for ER-directed breast cancer therapy include ER blockade using tamoxifen, a selective estrogen receptor modulator (SERM) with agonist and antagonost properties, or fulvestrant, a pure antagonist and selective estrogen receptor demodulator (SERD). ER-dependent tumor growth also can be targeted by removal of the estrogen agonist using aromatase inhibitors, which prevent peripheral and tumor conversion of androgens to estrogen, lowering estrogen levels. Endocrine therapy of breast cancer can be highly effective and is associated with fewer side effects than many alternate systemic treatments such as chemotherapy. Thus, when response is likely, endocrine therapy is often the preferred mode of systemic treatment. Clinical factors can predict likely response to endocrine therapeutics; these include a long disease-free interval, metastasis to nonvisceral sites, and high levels of ER in the tumor.[2–5] However, the presence of the ER is the most important determinant of response, and response is unlikely in the absence of sufficient tumor ER expression.[2] ER expression is routinely measured in clinical practice by in vitro assay of biopsy material. Although in vitro ER expression is the strongest predictor of response to hormonal treatment, it is far from perfect. In vitro ER expression predicts response to hormonal treatment in 30% to 70% of untreated patients; however, objective response is seen in only 7% to 21% of previously treated patients.[2–7]

For ER-expressing metastatic breast cancer, hormonal therapy is often first-line treatment.[6] Thus, knowledge of ER status of metastatic disease is critically important for the treatment of metastatic breast cancer. This valuable information is often difficult to obtain. ER expression at metastatic sites may not the same as the ER expression of primary disease because of phenotypic changes that occur in 20% to 35% of women with metastatic breast cancer.[8,9] Tissue sampling is essential but is a challenge because of disease heterogeneity and sampling error. Tissue sampling is especially problematic in bone, a common site of disease spread in breast cancer.[9] Biopsy of bone lesions can be associated with significant morbidity and sampling error and may show only normal bony elements reacting to the presence of tumor. Furthermore, decalcification may result in loss of ER epitopes, making immunohistochemical evaluation difficult, or may show presence of ER, which may be non functional.[10]

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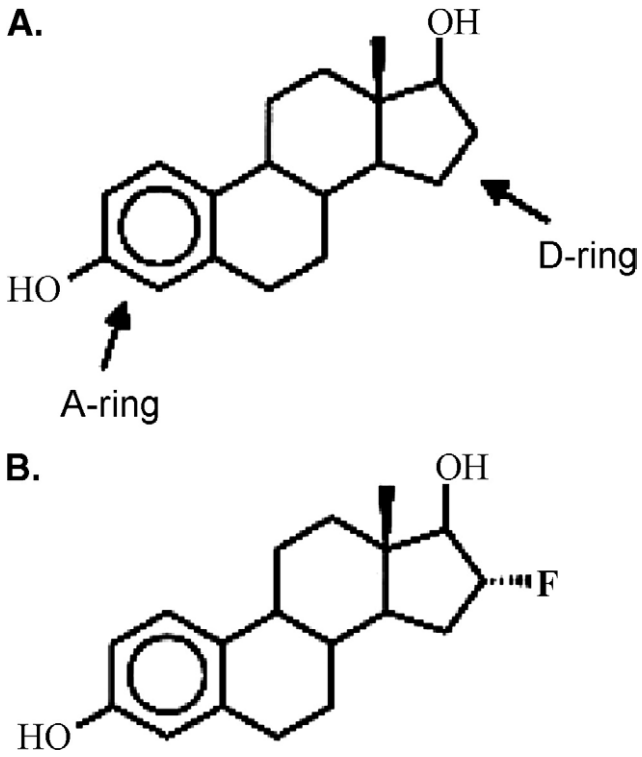

Figure 1 Structure of estradiol (A) and FES (B). Ring positions described in text are noted.

For all these reasons, alternative and complementary approaches to the determination of ER expression in advanced breast cancer would be clinically valuable.

Positron emission tomography (PET) with ER-targeting radiopharmaceuticals is a noninvasive method for assessing regional ER-expression in vivo that has emerging potential in providing answers to above mentioned challenges in treatment of metastatic breast cancer.[11] The advantages of in vivo assessment of estrogen receptors include avoiding sampling error and assessing the entire tumor volume receptor status rather than part of the tumor (addressing the heterogeneity of ER expression), and assessing the biological activity of the receptor at diagnosis and in response to treatment.

^{18}F-16α-17β-Fluoroestradiol (FES): Structure and Synthesis

A number of investigations of positron-emitting labeled estrogens, notably in the laboratory of Katzenellenbogen and colleagues,[12] led to the development of practical and effective ER-imaging agents for PET. Studies suggested that ^{18}F was an attractive label for PET ER imaging. Fluorine is a small halogen that can be substituted in several positions of the estrogen molecule without overly affecting chemical behavior.[13,14] Furthermore, ^{18}F has a sufficiently long half-life to permit multistep synthesis of ligands and uptake by target tissue and elimination by nontarget tissue during imaging. Early radiopharmaceutical development tested ^{18}F substituted in the D ring or 16-alpha position of hexestrol and demonstrated specific binding to immature rat uteri (Fig. 1).[15] Similar ^{18}F substitution for the steroidal analog estradiol showed highly selective uptake by target tissue with uterus-to-blood ratio of 39.[13] Although a variety of alternate agents have been tested and continue to be developed and tested,[12,14,16,17] the most successful ER imaging radiopharmaceutical to date is FES.[12,18]

Studies of different radiopharmaceuticals have yielded insights into the characteristics that make a good ER imaging agent. Some other compounds besides FES have been shown to have superior ER binding for in vitro assays and in vivo studies in rats. One such agent is ^{18}F-labeled moxesterol (^{18}F-betaFMOX), which demonstrated excellent results in preclinical models but performed poorly in human studies.[19,20] In studies comparing the metabolism of ^{18}F-FES and ^{18}F-betaFMOX,[20] immature rat hepatocytes were found to metabolize ^{18}F-FES 31 times faster than ^{18}F-betaFMOX, whereas mature rat hepatocytes metabolized ^{18}F-FES only 3 times faster, and baboon and human hepatocytes only 2 times faster than ^{18}F-betaFMOX. Thus, the very favorable target tissue uptake in rats of ^{18}F-betaFMOX may have been partially the result of species-specific resistance to metabolism. In addition, the estrogen transport protein, sex hormone binding globulin (SHBG), may play a role in determining the short-term uptake of radiopharmaceutical over the time scale of ^{18}F imaging and may also result in differences between rat and human biodistribution. Rats lack SHBG, whereas SHBG plays an important role in determining estrogen delivery to target sites in humans.[21] In rats, SHBG binding is not a factor, whereas in baboons and humans, ^{18}F-FES is extensively protein bound and protected from metabolism. Thus, in primates, SHBG may potentiate the ER-mediated uptake of ^{18}F-FES in ER-positive tumors by selectively protecting this ligand from metabolism and ensuring its delivery to receptor positive cells.[20] Ongoing studies and testing of new compounds may shed further light on this issue.[22–24]

Radiosynthesis of receptor-based agents can be more demanding than other nonreceptor PET radiopharacmeuticals in that high specific activity is necessary to assure that saturation by cold ligand does not limit uptake of the radiopharmaceutical in target tissue. In vitro and rodent studies suggest that less than 5 micrograms of FES should be injected for ER imaging[12]; however, this has not been well studied in humans.

The synthesis of FES has evolved somewhat since its development. The original synthesis developed by Kieswatter and colleagues[13] was subsequently optimized and automated to yield a radiopharmaceutical with high radiochemical purity and good specific activity at 90 minutes after end of bombardment (EOB).[25] An alternate synthesis developed by Lim and colleagues[26] started with more stable precursors and was highly successful in early patient studies at many centers, including ours. Subsequent refinements in the synthesis procedure used fewer steps and a single high performance liquid chromatography purification,[27] amenable to automated synthesis methods. Using this approach in our laboratory, we typically achieve a decay-corrected yield of 30% at 60 minutes EOB. Specific activity at 60 minutes after end of synthesis (EOS) ranges from 1 to 10 Ci/mmol, most typically 5 to 10

Ci/mmol, in which case, a 6-mCi injection of FES would result in <5 μg of FES injected even at injection times several hours after synthesis, permitting multiple patient studies from the same synthesis.

FES Pharmacokinetics and Safety

FES has binding characteristics similar to estradiol for both the ER and the transport protein, SHBG.[13,28] Typically in humans, approximately 45% of ^{18}F FES in circulating plasma is bound to SHBG, and much of the reminder is weakly bound to albumin.[28]

The clearance and metabolism of FES has been studied in both animals and humans.[29–32] Like other steroids, FES is highly extracted by the liver and, once injected, FES is rapidly taken up by the liver and metabolized. As a result, early blood clearance is rapid, reaching a plateau 20-30 minutes after injection.[29] By 20 minutes, only 20% of the circulating radioactivity is in the form of nonmetabolized FES. Glucuronide and sulfate conjugates of nonoxidized FES comprise most of the radiolabeled metabolites in blood. Metabolite excretion into the urine, mostly in the form of the glucuronide conjungates, occurs at a rate comparable with release from the liver. Underlying this finding is a pattern of enterohepatic circulation, where metabolites excreted into the bile are efficiently reabsorbed in the small intestine, with little radioactivity reaching the large intestine.[33] The total activity blood clearance curve declines slowly after 30 minutes after injection, with almost all of the circulating radioactivity in the form of labeled metabolites.[29] Because early FES clearance is rapid and metabolite background is nearly constant, imaging starting at 30 minutes after injection can provide good visualization of estrogen containing tissues, even in sites close to blood pool structures (Fig. 2).

To date, no toxicity or significant adverse reactions have been reported for ^{18}F-FES. Radiation dosimetry studies show organ doses with FES-PET are comparable with those associated with other commonly performed nuclear studies and potential radiation risks are well within acceptable limits. The effective dose equivalent is 80 mrem/mCi and the organ that received the highest dose was the liver at 470 mrad/mCi.[33] The recommended injection is 6 mCi or less.

FES-PET Imaging Methods

The FES PET imaging procedures published in the literature are similar but with some subtle variations. Dehdashti and coworkers[34,35] administered 6 mCi of FES, and approximately 90 minutes later, a 30-minute dynamic emission scan was obtained for the body region that included the lesion(s) of interest, followed by a transmission scan. Quantitative assessment was made using standardized uptake value (SUV), which is the decay corrected measurement per unit volume of tissue (μCi/mL) to the administered activity per unit of body weight (μCi/kg).

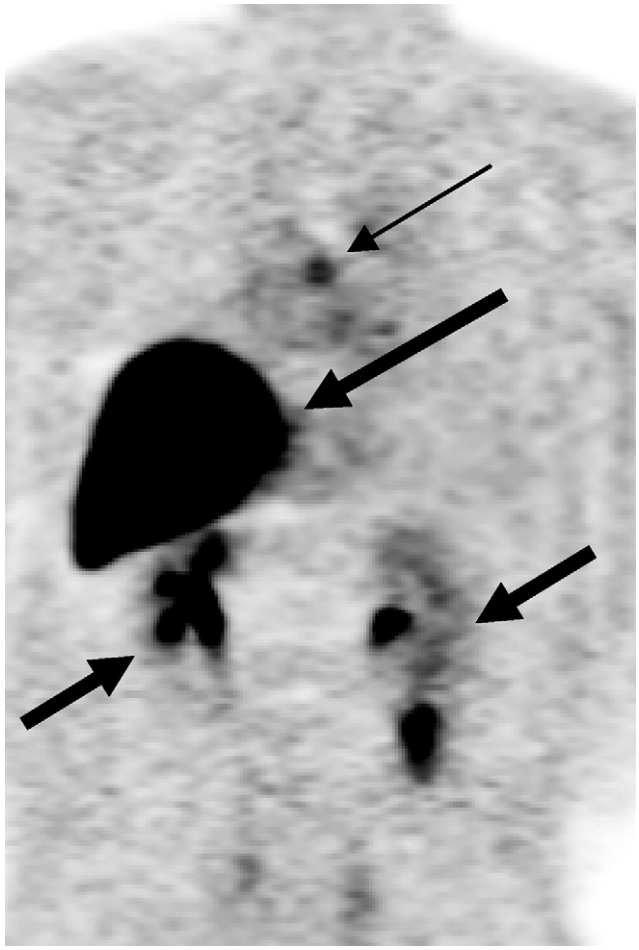

Figure 2 Coronal FES scans of a metastatic breast cancer patient showing prominent FES uptake in subcarinal nodal region (shown by thin arrows) and normal liver and kidney uptake (shown by thick arrows).

$$\mathrm{SUV} = \frac{\overline{C_t}}{\mathrm{ID}/\mathrm{wt}}$$

where $\overline{C_t}$ is the average tumor uptake from 90 minutes to 120 minutes after injection (μCi/mL), ID is the injected dose (mCi), and wt is the patient's body weight (kg).

Our group[36] has also used an injection of 6 mCi of FES and a dynamic emission scan from FES injection to 60 minutes over a single imaging field to capture FES blood clearance and tissue uptake kinetics. Blood clearance curves were calculated from dynamic imaging of the left ventricular cavity. Dynamic data collection was followed by a torso survey. SUV calculations reported in most data sets was similar to that used by Dehdashti and coworkers, but where the average tumor uptake was taken from 30 minutes to 60 minutes after injection. Changes in the blood clearance and tissue uptake curves after 60 minutes are slow and small, so SUVs reported by the St. Louis and Seattle groups are comparable. In addition our group has also explored the use of a measure of FES trapping termed Flux, which is calculated as

$$\text{Flux} = \frac{\bar{C}_t}{\int c_b(t)dt}$$

where $c_b(t)$ is the time varying blood curve. This measure has the advantage of accounting for variable blood clearance of FES. A similar approach was reported by Moresco for FES brain imaging studies.[31]

FES-PET Studies in Breast Cancer Patients

FES uptake has been validated as a measure of ER expression in breast tumors. Mintun and coworkers in 1988[37] showed an excellent correlation between FES uptake in the primary tumor measured on PET images and the tumor ER concentration measured in vitro by radioligand binding after excision in 13 patients with primary breast masses. Preliminary comparison of FES uptake to immunohistochenmistry assay of biopsy material from patient with both primary and metastatic cancer also showed a good correlation.[38]

In the earliest reported study of FES-PET in patients, FES uptake was observed at sites of primary carcinoma, axillary nodes, and one distant metastatic site.[37] The investigators then extended the use of this radiopharmaceutical for imaging of metastatic breast cancer. Sixteen patients with metastatic disease underwent FES-PET imaging with increased uptake seen on 53 of the 57 metastatic lesions resulting in a 93% sensitivity and only 2 apparent false positives.[39] Imaging results were reported quantitatively as percentage uptake of injected dose per mL, ratio of lesion to soft tissue, and as the ratio of lesion to uninvolved bone. The same group found similar results in a later study of FES imaging in 21 patients with metastatic breast cancer with 88% overall agreement between in vitro ER assays and FES-PET.[35] In addition to subjective analysis, FES uptake was reported as an SUV. Using an SUV $>$ 1 to identify ER-expressing disease, the sensitivity of FES imaging was 76% with no false positives in 21 metastatic breast cancer patients.[40]

A preliminary study of factors affecting average FES uptake at tumor sites was performed in 93 patients, 9 of which had primary breast cancer and 84 who had metastatic breast cancer. The results demonstrated significant associations between average SUV and menopausal status, serum estradiol levels, serum SHBG (SHBG levels) and previous hormonal therapy. Postmenopausal patients, patients with lower serum estradiol levels, patients with lower SHBG levels, and patients with prior hormonal therapy had greater average SUVs.[38] Follow-up analysis, including a larger heterogeneous population but with uniform subsets, is underway.

Heterogeneity of FES uptake as an indicator of heterogeneity of ER expression at metastatic sites has also been studied. Mortimer and coworkers[40] found that 4 of 17 (24%) patients with metastatic breast cancer had discordance in FES uptake between sites in individuals. Mankoff and coworkers[38] found an absence of FES uptake in one or more metastatic sites in 10% of patients who had primary ER-positive tumors. In this same preliminary study, the quantitative site-to-site variability in FES uptake in individuals was high (coefficient of variance of approximately 30%) and was not affected by the factors, mentioned previously, that influenced the average SUV. Thirteen percent of patients (6 of 47) with ER-positive primaries had one or more sites of FES-negative disease in a subsequent study by the same group.[36] The rate of loss of ER expression at metastatic sites from ER+ tumors is comparable, but slightly lower than, the literature based on tissue sampling,[8,9] suggesting that sampling error may contribute to apparent heterogeneity in tissue-based assay studies.

The primary use of in vitro ER assay in clinical practice is as a predictive assay for endocrine therapy. Although FES has not been prospectively tested as a predictive assay in clinical trials, comparison of FES uptake versus response to endocrine therapy in some groups of patients has yielded insights into the likely performance of FES-PET as a predictive assay (Fig. 3). Mortimer and coworkers[41] showed that the level of FES uptake predicted response to tamoxifen, demonstrating the potential utility of FES-PET in predicting response in the locally advanced and metastatic setting. Forty women with biopsy-proven ER-positive breast cancer had FES-PET before and 7 to 10 days after initiation of tamoxifen therapy and tumor FES-PET was assessed quantitatively with the SUV method. Percentage decrease in FES (responders = 55% ± 14%, nonresponders = 19% ± 17%), absolute change in tumor SUV (responders = 2.5 decrease ± 1.8, nonresponders = 0.5 decrease ± 0.6 SUV units) both predicted response to tamoxifen. The level FES uptake pretherapy also predicted response to tamoxifen. The positive and negative predictive value for baseline FES uptake using a arbitrarily selected cutoff of SUV of 2.0 were 79% and 88%, respectively.[41] No patient with an SUV less than approximately 1.5 responded.

Linden and coworkers[36] showed that initial FES uptake measurements in patients with ER-positive tumors was correlated with subsequent tumor response to 6 months of hormonal therapy. Forty-seven heavily pretreated patients with ER-positive metastatic breast cancer were given predominantly salvage aromatase inhibitor therapy. Objective response was found in 11 of 47 (23%) patients. FES-PET was assessed qualitatively and quantitatively using SUV and Flux calculations, as previously described. Although no patient without qualitative FES uptake at known tumor sites responded, qualitative FES-PET results did not significantly predict response to hormonal therapy. However, quantitative results were predictive of response in that 0/15 patients with initial SUV $<$1.5 responded to hormonal therapy, compared with 11/32 (34%) patients with initial SUV $>$1.5 ($P < 0.01$). Similar results were seen using FES Flux to measure uptake ($P < 0.005$). Interestingly, no patient whose tumor overexpressed HER-2 had an objective response, including patients with SUV $>$ 1.5. In the subset of patients without HER-2 overexpression 11/24 (46%) of patients with SUV $>$1.5 responded to hormonal therapy. Hypothetically, the use of FES-PET to select patients could have increased the response rate from 23% to 34% overall, and from 29% to 46% in the

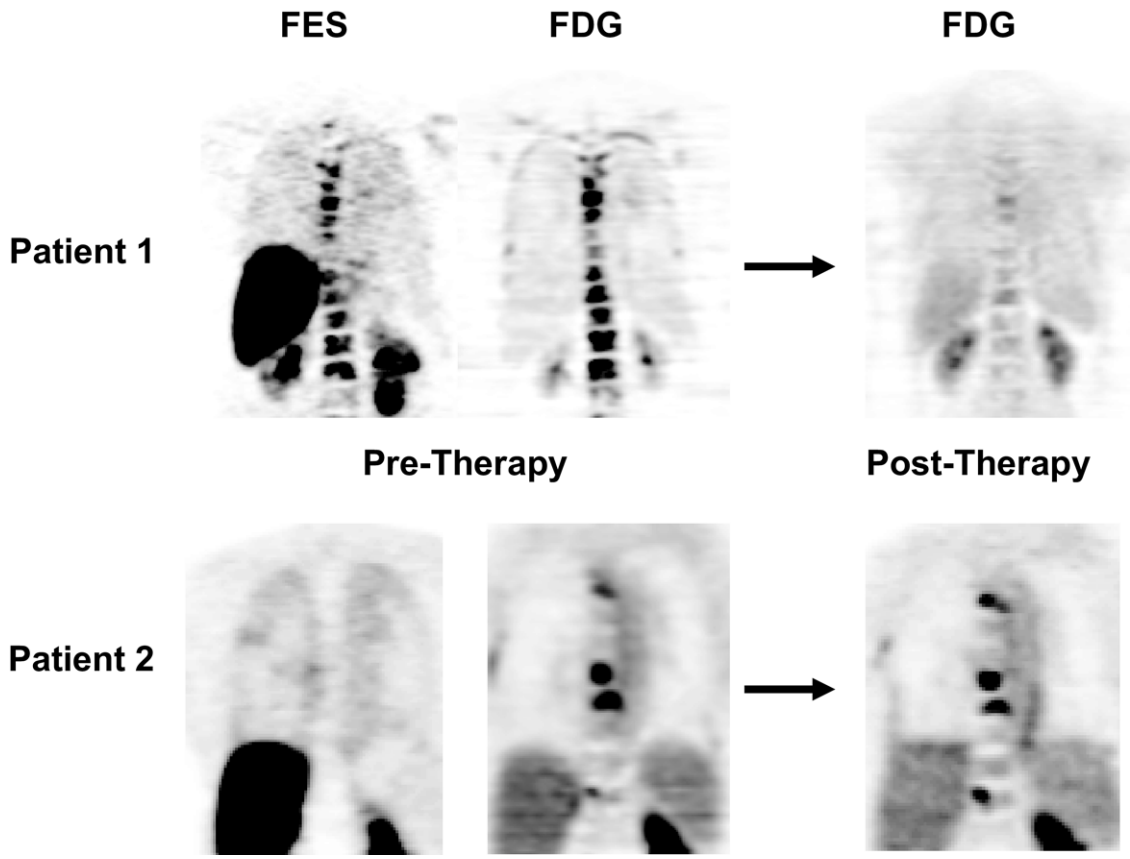

Figure 3 Two patients with documented bone metastases from an ER+ primary breast cancer imaged pre-hormonal therapy with FES (first column) and FDG (second column). Patient 1 has high FES uptake at all sites of active disease, indicating preserved ER expression. This patient subsequently responded to hormonal therapy (post-therapy scan in column 3). Patient 2 has no FES uptake at active sites of disease seen by FDG-PET, suggesting loss of ER expression, and had no response to hormonal therapy.

subset of patients lacking HER-2 overexpression. Timing of FES imaging may be a confounder in this study because patients underwent FES imaging while on aromatase inhibitor therapy but preliminary data from the same group shows that serial FES measurements change less than 20% in patients early after start of AI therapy.[42]

Serial FES-PET can be used to measure the affect of ER binding and ER expression for different endocrine therapies. McGuire demonstrated tamoxifen blockade of the ER in serial FES-PET scans in early patient studies.[39] Mortimer[41] later showed a lower level of blockade occurring as early as one week after starting tamoxifen. Linden and coworkers[42] analyzed serial FES-PET in patients treated with different agents with differing mechanisms of action in patients with metastatic disease undergoing treatment with tamoxifen (n = 2), AI (n = 14), fulvetrant (n = 5). Patients were imaged a median of 29 days after starting treatment. The decline in FES in SUV was greater for antagonists (tamoxifen and fulvestrant) versus AIs, which lower the agonist concentration but do not block the receptor. Interestingly, posttreatment qualitative FES scans showed complete blockage with tamoxifen but incomplete blockage with fulvestrant in 4 of the 5 patients, despite complete blockage of uterine uptake.

FES-PET Imaging in Other Tissues and Tumor Types

Some preliminary studies have evaluated FES-PET imaging in settings other than breast cancer. Moresco studied FES uptake in normal brain tissue and meningiomas,[31,32] using measures similar to the flux measure defined above. Although FES uptake in normal brain tissue was too low to quantify estradiol binding reliably by PET, significant FES uptake was seen in some meningiomas. Selective FES uptake by uterine endometrium has been shown in human imaging, with cyclic changes mirroring the menstrual cycle.[43] FES uptake in endometrial cancer has been reported for single patient studied by this method.[44] Preclinical studies have evaluated FES imaging of ER expressed as a marker protein as a potential method for monitoring gene therapy[45,46]; however, this has not been tested outside of early preclinical applications.

Future Directions

In addition to the promise of FES-PET, early studies point out some of the limitations of this method, including difficulty in

predicting disease stabilization in response to hormonal therapy, which can be an important clinical goal. All published studies to date used FES to study estrogen and ER pharmacology and biology, and were not designed as clinical trials. Future clinical trials should include prospective, multi-institutional, and with uniform selection criteria and treatment regimens. Given the importance of ER assay in current clinical trials and clinical practice of breast cancer, and promising early results with FES-PET, it is likely that these future studies will support FES-PET as a valuable tool for noninvasive ER assay for to guide drug development, clinical trials, and clinical practice.

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