Molecular imaging of estrogen receptors
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Chapter 2

PET imaging of estrogen receptors in patients with breast cancer

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ABSTRACT

Estrogen receptors (ER) are over-expressed in around 70% of all breast cancers and are a target for endocrine therapy. These receptors can be visualized on PET with 16α-[18F]-fluoro-17β-estradiol (18F-FES) as a tracer. Compared with biopsy, which enables assessment of individual sites, whole-body 18F-FES-PET enables quantification of ER expression in all metastases. In several studies, measurement of tumor protein ER expression by 18F-FES-PET, concurrent with biopsy, detected ER positive tumor lesions with a sensitivity of 84% and specificity of 98%. Roughly 45% of patients with metastatic breast cancer have heterogeneous ER expression, with 18F-FES positive and 18F-FES negative metastases. Low tumor 18F-FES uptake in metastases can predict failure of hormonal therapy in patients with ER positive primary tumors. Finally, 18F-FES-PET has shown that ER binding capacity changes after intervention with hormonal drugs, but findings need to be confirmed. Factors other than ER expression, including menopausal status and concomitant therapies, that can affect tumor 18F-FES uptake must be taken into account.
INTRODUCTION

Around 70% of all patients with breast cancer have ER positive tumors, which makes targeted endocrine therapy an attractive treatment option in adjuvant and metastatic settings. The success rate in breast cancer relies heavily on the tumor ER status, which is currently assessed by immunohistochemistry. Although immunohistochemistry is well suited to test primary breast tumors, accuracy is lower in metastases. ER expression can change over time, and discordant expression between primary tumors and metastases is seen in up to 40% of the patients. Biopsy might be useful to reassess a patient’s ER status, but is not always feasible. Moreover, heterogeneous ER expression can lead to sampling errors.

PET with 16α-[18F]-fluoro-17β-estradiol ([18F]-FES) enables non-invasive visualization and quantification of ER expression in all tumor lesions within a patient. In addition, this imaging technique can potentially provide in vivo information about ER binding of endocrine drugs. Insight into factors that might affect [18F]-FES uptake, such as endogenous estrogen concentrations and concurrent treatments, are relevant to the optimum use of this technique. In this review, we summarize the role of ER in breast cancer, address the potential of molecular imaging of ER expression, and discuss factors that potentially influence the uptake of [18F]-FES.

Estrogen receptors

When natural (e.g., estradiol) or synthetic ligands (e.g., ethynylestradiol, tamoxifen) bind to ER, dimerization and binding to specific DNA sequences occur and mediate the transcription of estrogen-responsive genes. There are two ER subtypes, ERα and ERβ, which are encoded by different genes (ESR1 and ESR2) and are located, respectively, on chromosomes 6q25.1 and 14q23.17 Being largely homologous in their DNA-binding domains (94%), both isoforms bind to similar DNA sequences. Differences in ligand-binding and activation function domains mean that ERα and ERβ can bind various ligands with different affinity and exert opposite actions. The role of ERβ, however, is not fully understood, and drugs that selectively target the ERβ isoform have not yet been studied in clinical trials of breast-cancer treatment. Therefore, in this review we focus on imaging of ERα (hereinafter referred to as ER).

Three classes of endocrine treatments are available that prevent the activation of ER by estrogens. Selective modulators competitively bind to ER and induce conformational changes that alter transcriptional activity. Aromatase inhibitors exert indirect effects on ER by inhibiting the conversion of androgens into estrogens. Finally, fulvestrant, a selective ER downregulator, competitively binds to and degrades receptors by increasing turnover rate.

The sole predictive factor for response to endocrine therapy is the tumor ER status. Patients
with even marginally ER positive tumors have a survival advantage.\textsuperscript{22} Patients with ER negative tumors are unlikely to benefit from endocrine therapy.\textsuperscript{23} Absence of progesterone receptors in ER positive tumors is suggested to be a marker of poor response to endocrine therapy, but might in fact be related to poor prognosis in progesterone receptor-negative disease. The benefit derived from endocrine therapy compared with placebo in patients with ER positive, progesterone receptor negative disease is similar to that in ER positive, progesterone receptor positive disease.\textsuperscript{21} Thus, progesterone receptor status has strong prognostic value but is of little predictive importance.

**Discordant ER expression**

ER status can switch from ER positive to ER negative and vice versa. In retrospective studies, discordant ER expression between the primary tumor and distant metastases was observed in 15–40\% of patients.\textsuperscript{24} Prospective data have shown loss of ER expression in distant metastases in three (12\%) of 25 patients with an ER positive primary tumor in one study,\textsuperscript{8} and in 11 (16\%) of 69 patients in another study.\textsuperscript{9} Gain of ER expression was observed in four (16\%) of 25 patients with an ER negative primary tumor.\textsuperscript{9} The clinical relevance of change in ER expression in metastases is supported by a retrospective study in 459 patients, in which ER expression in metastases predicted overall survival independent of the ER status of the primary tumor.\textsuperscript{25} The researchers concluded that patients in whom a status switches from negative to positive might benefit from endocrine therapy.

**PET imaging**

PET can be used to acquire functional information, such as ER expression, by use of ligands labeled with positron-emitting isotopes. \textsuperscript{18}F-FES is the preferred tracer for visualization of ER expression in clinical studies. This tracer is intravenously injected and allowed to accumulate in tissue expressing ER for around 60 min before PET is done. The accumulated \textsuperscript{18}F-FES decays by emitting a positron that annihilates after collision with an electron, which results in two $\gamma$-ray photons of 511 keV that travel in opposite directions. The PET camera detects these photons as they arrive simultaneously at opposite detectors.\textsuperscript{26} The events are converted into three-dimensional images by use of mathematical algorithms (figure 1).

PET images can be combined with CT or MRI to link PET findings to anatomical information. Additionally, multimodality scanners have become available, such as PET/CT and PET/MRI. These scanners acquire anatomical information about the location, density, and size of lesions simultaneously with the functional information obtained by PET. Current PET/CT cameras have an integrated CT camera that is between 16- and 256-slice, and a PET camera with a resolution as high as 2 mm. For whole body PET imaging, cameras scan between 1 and 3 minutes per bed position. Images from seven to eight bed positions (skull
to mid-thigh) are obtained. In whole body PET studies, tracer uptake is typically quantified as the standardized uptake value (SUV), which is calculated as radioactivity in the volume of interest (kBq/ml) divided by the injected dose per kg bodyweight (MBq/kg). SUV can be affected by the partial volume effect, which is especially prominent in lesions with sizes that approach the spatial resolution of the PET camera. MRI and CT can correct for this effect. Accuracy of ER density measurements can be improved by kinetic modeling studies. These studies however require arterial blood sampling, analysis of 18F-FES metabolism and dynamic imaging during the course of 60-90 minutes in one bed position, which precludes whole body imaging. Thus, kinetic modeling studies are not feasible in routine clinical practice, but should be done to validate SUV for ER expression.

PET tracers
The most potent endogenous ER agonist is estradiol (figure 2A). Only a small percentage (1–3%) of the total estradiol circulates in the biologically active form; most is bound to plasma carrier proteins, such as sex hormone binding globulin (SHBG) and albumin. The development of PET tracers that target the ER has focused on radio-labeling of estradiol and structural analogues. Around 20 18F-labeled estrogen analogues have been described. Of these, 18F-FES (figure 2B) is the most extensively characterized and is most frequently used in clinical studies (supplemental files). 18F-FES has a 60–100% relative binding affinity for ER. Affinity for ERα is 6.3-fold higher than that for ERβ, and greater specificity for the ERα isoform has been shown in vivo in knockout mouse studies. Binding characteristics are affected by substituents and by the position of the 18F-atom. For example, the addition of ethynyl at the 11β- or 17α-position of 18F-FES increases ER binding affinity, but 11β-methoxy substitution decreases affinity. The increased affinity of ethynyl estrogens for ER coincides with raised non-specific binding owing to increased lipophilicity.

In animal studies, various ER tracers showed specific uptake in the uterus and ovaries that
could be blocked by the addition of unlabelled estradiol.\textsuperscript{33} In mice, ER positive murine mammary adenocarcinomas specifically take up \textsuperscript{18}F-FES, whereas uptake is absent in ER knock-down tumors.\textsuperscript{34} In rats with carcinogen-induced mammary tumors, \textsuperscript{18}F-FES uptake is seen in tumor lesions, which correlates with \textit{ex vivo} quantitative measurements of ER concentrations.\textsuperscript{35,36}

All radiolabeled estrogens are rapidly metabolized in the liver and excreted via the gastrointestinal tract in bile and via the kidneys in urine. To decrease the rate of metabolism, substituents can be added at the A-ring, where most of the conjugation of \textsuperscript{18}F-FES occurs. In rats, an \textsuperscript{18}F atom in the A-ring of \textsuperscript{18}F-FES resulted in lower liver uptake and slightly increased uterus-to-blood ratios, which was ascribed to decelerated metabolism.\textsuperscript{37}

Whether the tracer’s affinity for SHBG affects tumor uptake is not entirely clear. SHBG regulates the bioavailability of steroids and, therefore, tracers with low affinity for SHBG were suggested for ER-targeted PET.\textsuperscript{38,39} In 239 patients with metastatic breast cancer, uptake of \textsuperscript{18}F-FES in tumors decreased with increasing SHBG concentrations and binding of \textsuperscript{18}F-FES to SHBG.\textsuperscript{40} Paradoxically, \textsuperscript{18}F-fluoro-moxestrol, an ER tracer with 256-fold lower binding affinity for SHBG compared to \textsuperscript{18}F-FES, did not improve results in a clinical study. Excellent ER mediated uptake was seen in rodents that lack SHBG, but in a later clinical study, none of three ER positive primary breast tumors were visualized by \textsuperscript{18}F-fluoro-moxestrol PET.\textsuperscript{41,42} These findings could not be explained by resolution limitations of the PET camera,

Figure 2. 17β-estradiol consists of four cycloalkane rings and two hydroxyl groups. The numbers indicate commonly used positions for substituents (A). 16α-[\textsuperscript{18}F]-17β-estradiol is the currently preferred ER tracer (B).
because the tumors were larger than 15 mm. To ensure delivery of the tracer to tumors in human beings, therefore, ER tracers may need good binding affinity for SHBG.

Development of other ER tracers deserves further exploration. For PET imaging, those that are less extensively cleared by the liver might improve visualization of liver metastases. ER tracers with increased binding affinity may well improve tumor-to-background ratios.\textsuperscript{43} Labeling of PET tracers specific for the ER\textsubscript{\textbeta} isoform might be of interest in other tumor types.\textsuperscript{44} Single-photon emission computed tomography (SPECT), which is widely available with \textsuperscript{99m}Technetium and iodine labeled ER ligands, have been assessed in small clinical studies.\textsuperscript{45–47} Finally, non-radioactive ER tracers for use in MRI or near-infra-red fluorescent optical imaging to guide surgery could be of clinical use.\textsuperscript{48}

\textsuperscript{18}F-FES is deemed an investigational new drug and, therefore, relevant documents (available at: http://imaging.cancer.gov/programsandresources/fes-documentation) might be needed for its use in clinical trials. Quality control tests and modifications might also be needed to match local requirements.

**Clinical studies**

Multiple studies of \textsuperscript{18}F-FES-PET have been done in several countries (Italy, Japan, the Netherlands, and the USA; table 1).\textsuperscript{10,16,40,49–68} Thirteen ongoing \textsuperscript{18}F-FES-PET studies in breast cancer plus one in ovarian cancer are registered with ClinicalTrials.gov – seven in the USA, three in the Netherlands, two in Canada, one in France, and one in South Korea. \textsuperscript{18}F-FES-PET studies have also been reported in endometrial cancer,\textsuperscript{54,55,61} uterine tumors,\textsuperscript{49,51,59} meningiomas,\textsuperscript{68} and ovarian cancer.\textsuperscript{56}

**\textsuperscript{18}F-FES-PET in healthy volunteers**

Typically, 200 MBq \textsuperscript{18}F-FES is injected before PET, with a specific activity higher than 25,000 GBq/mmol, which comprises an injected mass of less than 8 nmol \textsuperscript{18}F-FES. \textsuperscript{18}F-FES is initially metabolized rapidly, and less than 20% of the injected dose remains circulating as unconjugated \textsuperscript{18}F-FES after 10 min. Glucoronide- and sulfatase-conjugated \textsuperscript{18}F-FES is excreted via the gut in bile. As a result of enterohepatic circulation, only a low amount of \textsuperscript{18}F-FES enters the colon. The reabsorbed conjugated \textsuperscript{18}F-FES is cleared mainly by the kidneys. After the initial rapid decline, the activity of unconjugated \textsuperscript{18}F-FES in blood declines slowly over the next 50 min and reaches less than 5% of peak values after 60 min. The presence of conjugated \textsuperscript{18}F-FES metabolites is unlikely to affect tumor \textsuperscript{18}F-FES uptake because they cannot bind ER and their polarity prevents penetration of cell membranes.\textsuperscript{35,69} Unconjugated \textsuperscript{18}F-FES is bound to carrier proteins SHBG (~45%) and albumin (~45%) in serum and the remainder circulates in free form.\textsuperscript{66}
### Table 1: Overview of published clinical \(^{18}\)F-FES-PET studies

<table>
<thead>
<tr>
<th>Year</th>
<th>Condition</th>
<th>n</th>
<th>Study aim</th>
<th>Conclusion</th>
<th>Ref #</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>Uterine tumors</td>
<td>47</td>
<td>To evaluate the relationship between tumor (^{18})F-FDG and (^{18})F-FES uptake with ER, GLUT-1, and Ki-67</td>
<td>(^{18})F-FES uptake was correlated with ERα and PR levels and (^{18})F-FDG uptake with GLUT-1 and Ki-67</td>
<td>49</td>
</tr>
<tr>
<td>2012</td>
<td>Breast cancer</td>
<td>33</td>
<td>To determine clinical value of (^{18})F-FES-PET in patients with a diagnostic dilemma unresolved by conventional techniques</td>
<td>(^{18})F-FES-PET, as add-on to conventional techniques, can aid diagnosis and therapy decision-making in breast cancer patients with a diagnostic dilemma</td>
<td>50</td>
</tr>
<tr>
<td>2011</td>
<td>Breast cancer</td>
<td>239</td>
<td>To examine correlations between (^{18})F-FES uptake and clinical and laboratory data, prior treatments and (^{18})F-FES metabolism</td>
<td>(^{18})F-FES uptake was positively correlated with body mass index and inversely correlated with plasma SHBG levels and %binding</td>
<td>40</td>
</tr>
<tr>
<td>2011</td>
<td>Suspected uterine sarcoma</td>
<td>24</td>
<td>To investigate value of (^{18})F-FES-PET in addition to (^{18})F-FDG-PET to differentiate between uterine sarcoma and leiomyoma</td>
<td>(^{18})F-FDG/(^{18})F-FES ratios &gt; 2.0 differentiated between sarcoma and myoma with a 90.9% sensitivity and 92.3% specificity</td>
<td>51</td>
</tr>
<tr>
<td>2011</td>
<td>Metastatic breast cancer</td>
<td>91</td>
<td>To measure inter-patient and within-patient (site-to-site) variability in (^{18})F-FES uptake</td>
<td>(^{18})F-FES uptake varied greatly between patients. 37% of patients had low/absent (^{18})F-FES uptake. A subset of patients had mixed uptake</td>
<td>52</td>
</tr>
<tr>
<td>2011</td>
<td>Metastatic breast cancer</td>
<td>30</td>
<td>To measure changes in (^{18})F-FES uptake during aromatase inhibitors, tamoxifen and fulvestrant</td>
<td>(^{18})F-FES uptake is not affected by aromatase inhibitors and decreases by ~55% for tamoxifen and fulvestrant</td>
<td>53</td>
</tr>
<tr>
<td>2009</td>
<td>Endometrial cancer</td>
<td>19</td>
<td>To evaluate correlation between (^{18})F-FES-PET, (^{18})F-FDG-PET and ER status by IHC</td>
<td>(^{18})F-FES/(^{18})F-FDG ratios are correlated to tumor ERα status</td>
<td>54</td>
</tr>
<tr>
<td>2009</td>
<td>Endometrial cancer</td>
<td>31</td>
<td>To evaluate correlation between (^{18})F-FES-PET, (^{18})F-FDG-PET and clinicopathologic features</td>
<td>(^{18})F-FES/(^{18})F-FDG ratios are correlated to tumor aggressiveness</td>
<td>55</td>
</tr>
<tr>
<td>2009</td>
<td>Ovarian cancer</td>
<td>3</td>
<td>To review role of PET in ovarian cancer (+ preliminary results of (^{18})F-FES-PET in 3 patients)</td>
<td>(^{18})F-FES uptake was present in patients with ER positive tumors</td>
<td>56</td>
</tr>
<tr>
<td>2009</td>
<td>Metastatic breast cancer</td>
<td>59</td>
<td>To determine whether (^{18})F-FES-PET and serial (^{18})F-FDG-PET (+estradiol challenge) can predict response to endocrine therapy</td>
<td>Baseline tumor (^{18})F-FES uptake and metabolic flare after estradiol challenge are both predictive of responsiveness to endocrine therapy</td>
<td>57</td>
</tr>
<tr>
<td>2008</td>
<td>Primary and metastatic breast cancer</td>
<td>17</td>
<td>To correlate (^{18})F-FES uptake with IHC</td>
<td>(^{18})F-FES uptake showed good correlation with IHC for ERα</td>
<td>58</td>
</tr>
<tr>
<td>2008</td>
<td>Endometrial hyperplasia</td>
<td>2</td>
<td>Case report on influence of tamoxifen on (^{18})F-FES uptake</td>
<td>The use of (^{18})F-FES-PET to evaluate ER status requires careful attention regarding the influence of endocrine therapy</td>
<td>59</td>
</tr>
<tr>
<td>2008</td>
<td>Benign and malignant uterine tumors</td>
<td>38</td>
<td>To evaluate relationship between (^{18})F-FES uptake and (^{18})F-FDG uptake in benign and malignant uterine tumors</td>
<td>(^{18})F-FES/(^{18})F-FDG ratio could provide pathological information for differential diagnosis of uterine tumors</td>
<td>57</td>
</tr>
</tbody>
</table>
PET imaging of estrogen receptors

2007 Healthy volunteers 16 To evaluate relation between 18F-FES uptake, menstrual cycle and endogenous estrogen levels
Changes in 18F-FES uptake were consistent with changes in ER levels determined by studies using IHC.

2007 Endometrial cancer 1 Case report on use of 18F-FES-PET to evaluate response to medroxyprogesterone in endometrial cancer
18F-FES-PET, but not 18F-FDG-PET, showed remaining focal uptake, confirmed by histology.

2007 Metastatic breast cancer 1 Case report about 18F-FES-PET and 18F-FDG-PET findings in a patient with gastric limiites plastica from lobular breast cancer metastases
18F-FES-PET confirmed regional ER binding of lobular ER positive breast cancer metastases.

2006 Metastatic breast cancer 47 To quantify tumor 18F-FES uptake as predictor of response to endocrine therapy
Absence of 18F-FES uptake predicts failure of endocrine therapy.

2001 Breast cancer 40 To evaluate serial 18F-FES-PET and 18F-FDG-PET to predict response to tamoxifen
Increase in 18F-FDG uptake and decrease in 18F-FES uptake after the initiation of tamoxifen is predictive of response.

2001 Breast cancer 49 Radiation dosimetry
Radiation dose is comparable to commonly used nuclear medicine tests.

1999 Breast cancer 18 To evaluate the interaction between SHBG and 18F-FES
45% of 18F-FES is bound to SHBG, which is likely to affect tracer uptake.

1999 Metastatic breast cancer 11 To evaluate serial 18F-FES-PET and 18F-FDG-PET to predict response to tamoxifen
Increase in 18F-FDG uptake and decrease in 18F-FES uptake after the initiation of tamoxifen is predictive of response.

1997 Meningioma 6 To evaluate ER status of meningiomas by means of 18F-FES-PET
Four of six patients showed focal 18F-FES uptake. 18F-FES uptake correlated to IHC status in five of six patients.

1997 Primary or metastatic breast cancer 15 To evaluate clearance of labelled 18F-FES metabolites
18F-FES clearance is rapid.

1996 Primary or metastatic breast cancer 43 To assess the correlation between 18F-FES-PET and 18F-FDG with in vitro assays, and response to therapy
18F-FES-PET had a sensitivity of 76% and specificity of 100% when compared to IHC.

1995 Primary or metastatic breast cancer 53 To compare 18F-FES-PET with 18F-FDG-PET and IHC
Overall agreement between 18F-FES-PET and IHC 88%, providing non-invasive information that cannot be obtained by 18F-FDG-PET.

1991 Metastatic breast cancer 16 To evaluate use of 18F-FES-PET in ER+ metastatic postmenopausal breast cancer patients
18F-FES-PET sensitivity for metastases was 93%.

1989 Primary breast cancer 13 Feasibility of 18F-FES-PET to detect primary ER+ breast tumor lesions and correlation with in vitro ER status
18F-FES-PET showed focal uptake in all patients. 18F-FES uptake correlated well with in vitro assays (r=0.96).

n = number of patients studied; Ref# = reference number
The radiation burden for the patient is 0.022 mSv/MBq, resulting in 4.4 mSv per 200 MBq 18F-FES injection. The organs exposed to the highest radiation doses are the liver (0.13 mGy/MBq), gallbladder (0.10 mGy/MBq) and bladder (0.05 mGy/MBq). ER-specific uptake has been observed in the uteri of healthy premenopausal volunteers with a SUV of roughly 2.5 in the myometrium and 4.0–6.0 in the endometrium. Endometrial SUVs were higher in the proliferative phase of the menstrual cycle compared to the secretory phase.

**Sensitivity and specificity**

Four studies involving 114 patients have assessed sensitivity and specificity of 18F-FES-PET for ER positive breast cancer lesions concurrently with in vitro assay for ER expression (table 2). Sensitivity was good overall and quantitative 18F-FES uptake correlated moderately to excellently with ER expression (r = 0.56–0.96). Metastases can be seen with 18F-FES-PET in multiple sites, such as in the liver, bone, lung and lymph nodes, while physiological background is present in liver, bile duct, intestinal tract, and bladder (figure 3). In one study, 18F-FES-PET visualized only 6 out of 10 ER positive primary breast tumors, although the four patients with false-negative findings were aged 34–45 years and were probably premenopausal; the ages of the six patients with true-positive 18F-FES-PET scans ranged from 56 to 71 years. Premenopausal patients might have impaired 18F-FES uptake because of competitive binding by endogenous estrogens, but this hypothesis needs to be tested in a larger sample. Other studies included either only postmenopausal patients or have not calculated the correlation between menopausal status and 18F-FES-PET results. As well as variations in selection criteria for patients, the studies used different PET cameras, definitions of 18F-FES positivity, and methods of quantification (tumor-to-background ratio vs SUV).

<table>
<thead>
<tr>
<th>Author</th>
<th>Biopsy</th>
<th>Sensitivity/specificity</th>
<th>Lesions (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% (% 95% CI)</td>
<td></td>
</tr>
<tr>
<td>Dehdashti71</td>
<td>ER+ tumor</td>
<td>69% (44-86%)</td>
<td>16</td>
</tr>
<tr>
<td>Mintun</td>
<td>ER+ tumor</td>
<td>100% (77-100%)</td>
<td>13</td>
</tr>
<tr>
<td>Mortimer</td>
<td>ER+ tumor</td>
<td>76% (55-89%)</td>
<td>21</td>
</tr>
<tr>
<td>Peterson</td>
<td>ER+ tumor</td>
<td>100% (76-100%)</td>
<td>12</td>
</tr>
<tr>
<td>Overall sensitivity</td>
<td></td>
<td>84% (73-91%)</td>
<td>62</td>
</tr>
<tr>
<td>Dehdashti71</td>
<td>Benign</td>
<td>100% (68-100%)</td>
<td>10</td>
</tr>
<tr>
<td>Mortimer</td>
<td>ER- tumor</td>
<td>100% (82-100%)</td>
<td>17</td>
</tr>
<tr>
<td>Peterson</td>
<td>ER- tumor</td>
<td>100% (84-100%)</td>
<td>20</td>
</tr>
<tr>
<td>Overall specificity</td>
<td></td>
<td>80% (38-96%)</td>
<td>5</td>
</tr>
<tr>
<td>Peterson</td>
<td>ER- tumor</td>
<td>98% (90-100%)</td>
<td>52</td>
</tr>
</tbody>
</table>
Chapter 2

PET imaging of estrogen receptors

High specificity of $^{18}$F-FES for ER is illustrated by the absence of $^{18}$F-FES uptake in benign lesions and ER negative breast cancer lesions. Combined data from three studies showed that 51 of 52 histologically benign or ER negative lesions were also $^{18}$F-FES negative (SUV below 1.0) and, therefore, overall specificity is 98% (table 2).10,70,71

Prediction of treatment response

Four studies reported the predictive value of $^{18}$F-FES uptake by tumors for response to endocrine therapy in 138 patients with metastatic breast cancer.57,63,64,67 All patients initially had ER positive primary tumors. $^{18}$F-FES-PET examination was done before a new line of endocrine therapy was started: 76 received aromatase inhibitors, 45 tamoxifen, and 17 fulvestrant. In one study, tamoxifen was discontinued at least 60 days before $^{18}$F-FES-PET to avoid competitive binding to the ER by tamoxifen and its metabolites. In the other studies, drug-free periods were not reported. Thresholds of 1.5 and 2.0 tumor $^{18}$F-FES SUV to select patients for endocrine therapy were assessed. Sixty-seven (49%) of 138 patients who received endocrine therapy showed clinical benefit according to response evaluation criteria in solid tumors (objective tumor response or stable disease for 6 months or longer) or clinical assessment. If the 1.5 threshold for tumor $^{18}$F-FES SUV would have been used, 96 would have received treatment, of whom 62 would have experienced clinical benefit (positive predictive value 65%). In 42 patients with tumor $^{18}$F-FES SUV lower than 1.5, 37 showed no clinical benefit from endocrine therapy (negative predictive value 88%). Hence, in patients with a previously ER positive primary tumor histology, tumor $^{18}$F-FES SUV below 1.5 predicts failure to respond to endocrine therapy. If denial of endocrine treatment had been based on tumor $^{18}$F-FES SUV threshold of 2.0 instead of lower than 1.5, a notable

Figure 3. Physiological $^{18}$F-FES uptake can be observed in liver, bile duct, intestinal tract and bladder. A limited number of ER positive bone, liver and nodal metastases are indicated.
proportion of patients who would have responded to treatment would not have received it (19 [31%] of 62, figure 4). Larger studies are needed to determine the predictive value of $^{18}$F-FES-PET. So far, differences between studies in predictive values for response to endocrine therapy and cutoff values, and the small number of patients studied have led to low-level evidence (level 3).\textsuperscript{72}

Assessment of heterogeneous ER expression

One of the advantages of $^{18}$F-FES-PET is that it provides a whole-body indication of ER expression across metastases. Most studies have so far used a cutoff SUV of 1.5 to classify $^{18}$F-FES positive and $^{18}$F-FES negative results. With use of this threshold, up to 37\% of patients with a previously ER positive primary breast tumor develop $^{18}$F-FES negative metastatic disease.\textsuperscript{52} Additionally, in three studies comparing $^{18}$F-FES-PET with 2-deoxy-$^{[18}$F]fluorodeoxyglucose PET in 107 patients, 15–47\% of the patients had $^{18}$F-FES-positive and $^{18}$F-FES-negative tumor sites.\textsuperscript{70,71,73} Another study showed no $^{18}$F-FES uptake in one or more metastases in ten (45\%) of 22 patients with at least one $^{18}$F-FES-positive lesion.\textsuperscript{50} Furthermore, heterogeneous ER expression is supported by up to a 6-fold difference in quantitative tumor $^{18}$F-FES uptake across metastases within an individual, and has a high coefficient of variance (30–68\%).\textsuperscript{50,52,74}

These data strongly suggest site-to-site variability in ER expression across metastases within individual patients. Early results of a phase 2 study in 15 patients with newly diagnosed metastatic breast cancer showed progressive disease in three (75\%) of four patients with at least one metastasis with visually absent $^{18}$F-FES uptake. By contrast, eight (73\%) of
11 patients (73%) without heterogeneous sites showed clinical benefit from endocrine therapy. Final results of this study and a prospective study are awaited (NCT00602043 and 00358098).

In vivo ER binding
In two studies, a change in $^{18}$F-FES uptake during endocrine therapy was investigated as an early predictor of treatment response. In a prospective study in 40 postmenopausal patients with locally advanced and metastatic breast cancer, a decrease in $^{18}$F-FES uptake was seen 7–10 days after tamoxifen was started. Patients with clinical benefit had greater decreases in $^{18}$F-FES uptake (55% [± 14%]) than did non-responding patients (19% [± 17%]). Whether the 7–10 day period is the optimum time to assess the effects of tamoxifen on ER is unclear, since concentrations of tamoxifen metabolites take 4–6 weeks to become stable. Nevertheless, the results are interesting because response to endocrine therapy can take several months to show accurately by anatomical imaging methods.

A retrospective study of $^{18}$F-FES-PET in patients with metastatic breast cancer receiving fulvestrant (n=11) or tamoxifen (n=5) showed a 49% and 55% decrease in $^{18}$F-FES uptake from baseline, respectively during treatment. Tumor $^{18}$F-FES uptake was decreased more with tamoxifen than with fulvestrant on the basis of post-treatment SUV lower than 1.5 (tamoxifen five of five patients vs fulvestrant four of 11 patients; $P=0.019$). Fulvestrant, however, was given as 250 mg intramuscularly (with or without a loading dose of 500 mg), rather than 500 mg every 4 weeks plus a 500 mg loading dose on day 14, which has since been approved. The inadequacy of fulvestrant 250 mg to block tumor $^{18}$F-FES uptake remains of interest, however, in light of the improved efficacy of the 500 mg dose. A trial in 16 patients was performed to investigate residual binding capacity during treatment with the 500 mg fulvestrant regimen (see chapter 4). $^{18}$F-FES-PET could offer the opportunity to assess binding of novel ER antagonists in early clinical trials and at various dose levels.

Absolute and relative decrease in $^{18}$F-FES uptake by ER antagonists varies widely, which could be related to variability in serum and tumor drug concentration and conversion of the drugs into more potent metabolites. For instance, tumor concentrations of the tamoxifen metabolite hydroxytamoxifen ranged between 0.4 and 564.5 ng/g in patients with ER positive tumors treated with 20 mg tamoxifen daily for 28 days. Monitoring of drug pharmacokinetics together with molecular imaging could provide insight into the relevance of drug concentrations to achieve sufficient ER binding in the tumor. Adequately powered prospective studies are warranted to find out whether serial $^{18}$F-FES-PET has a role during drug development or in the clinic as an early predictor of treatment response.
Use of $^{18}$F-FES-PET during treatment with aromatase inhibitors

Treatment with aromatase inhibitors would theoretically be expected to increase tumor $^{18}$F-FES uptake by reducing competitive binding, but in contrast a slight decrease (13%) was detected soon after the start of treatment in a retrospective study of 14 patients. A potential explanation for this finding is that a plateau of $^{18}$F-FES binding had already been reached by the time of assessment. Thus, an increase in available ER binding sites might not result in an increased maximum SUV. Moreover, results might be different after long-term exposure to aromatase inhibitors. Indeed, in the same study patients who started fulvestrant had higher baseline tumor $^{18}$F-FES uptake than other patients (tumor $^{18}$F-FES SUV 3.8 vs. 2.3), and, of these 11 patients, ten were already taking chronic aromatase inhibitor treatment when baseline $^{18}$F-FES-PET was done. Theoretically, therefore, this increased $^{18}$F-FES uptake could be the consequence of an increase in the percentage of free ER binding sites, or an absolute increase in ER expression, for instance as a cellular response to long-term estrogen-deprivation.

Timing of $^{18}$F-FES-PET in relation to concomitant therapies

Since ER antagonists clearly affect tracer uptake, most studies require a drug-free period of 6-8 weeks before baseline quantitative measurements are taken. Whether this is sufficient to completely eliminate competitive binding is unknown, particularly for fulvestrant, which has a half-life of 40 days and both blocks and degrades ER. A longer drug-free period, therefore, might be needed. Moreover, tumor drug concentrations could remain high for a long period despite normal concentrations in plasma. Several drug metabolites should also be taken into account (especially for tamoxifen). More work is required to define the most optimum times to do $^{18}$F-FES-PET.

Effects on tumor $^{18}$F-FES uptake

Tumor-cell-specific mechanisms

Endocrine resistance, which can be intrinsic or acquired, is a common complication. Resistance can occur with lost, decreased, or preserved ER expression. Lost or decreased ER expression might be caused by various mechanisms. Among them are ER negative cancer stem cells that drive metastases formation, clonal selection of endocrine resistant cells, and epigenetic changes. The fact that low or absent $^{18}$F-FES uptake is a good predictor of endocrine resistance in clinical studies makes this method relevant to assess drug resistance.

Endocrine resistance with preserved ER expression can be the result of ligand-independent activation of the ER via cross-talk with other receptors, such as EGFRs and the downstream mTOR. In case of a preserved ligand binding domain, $^{18}$F-FES will still bind to tumor ER.
In these instances, ER degradation by fulvestrant may remain effective. Endocrine therapy combined with other targeted therapies might also by-pass ligand-independent activation of the ER: mTOR inhibition combined with aromatase inhibition has shown promising results in patients with endocrine-resistant breast cancer. Finally, mutations in the ER gene can lead to alterations in function and ligand binding affinity. When point mutations are artificially introduced in the ligand domain, such as L536N and G525L, ligand-binding affinity is strongly reduced in vitro. This would also lead to reduced ¹⁸F-FES uptake. Other mutations, such as K303R, have been identified in breast cancer patients, and have been associated with resistance to endocrine therapy in preclinical models. Further studies are needed to address the relevance and frequency of ER mutations during disease progression.

Several mechanisms of endocrine resistance are purported, with various hypothesized effects on tumor ¹⁸F-FES uptake and possible consequences for treatment (figure 5). Serial ¹⁸F-FES-PET might provide some insight into changes in ER expression during disease progression and how endocrine resistance develops and, in combination with tumor biopsy and detailed genotyping, improve the selection of patients for treatment.

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*Figure 5.* Potential mechanisms of resistance to ER-targeted therapy, expected ¹⁸F-FES-PET results and consequence for therapy. ER positive tumor cells are indicated in blue and ER negative cells in green.
**Menopausal status**

Endogenous estrogens should theoretically lessen tracer uptake by competitive binding. Owing to ER driven uptake, estradiol concentrations are up to ten times higher in ER positive tumors than in plasma. Furthermore, intra-tumor estradiol levels correlate with those in plasma, which suggests that tumor estradiol concentrations are higher in premenopausal patients than in postmenopausal patients. Indeed, concentrations of estradiol within the mastectomy tissue of 47 patients with ER positive breast cancer were six times higher in premenopausal patients than in postmenopausal patients (1621.8 vs. 267.1 fmol/g).

$^{18}$F-FES-PET sensitivity seemed to be poor in a study that included premenopausal patients. Despite this observation, whether endogenous estrogen concentrations are sufficiently high to have a notable effect on tumor $^{18}$F-FES uptake is unclear. A retrospective study found no significant association between serum estradiol concentrations and tumor $^{18}$F-FES uptake.

Furthermore, neither low specific activity of $^{18}$F-FES nor higher injected mass of unlabelled estradiol were associated with decreased tumor $^{18}$F-FES uptake, which suggests that the exogenous dose of $^{18}$F-FES does not saturate the ER. Finally, in 14 patients treated with aromatase inhibitors, which was expected to increase $^{18}$F-FES uptake by reducing competitive binding, serial $^{18}$F-FES-PET imaging did not show such an effect. The absence of concurrent biopsies, and the broad variability between patients in estradiol concentrations, tumor ER expression and injected mass of $^{18}$F-FES, makes precise analysis of the effects of background estradiol concentrations in individual patients difficult.

**Body composition**

$^{18}$F-FES is lipophilic and, therefore, patients with increased fat mass could be expected to have lower tumor $^{18}$F-FES uptake than leaner patients because of an increased pharmacological distribution volume, as suggested by animal studies. Additionally, raised concentrations of estrogen in serum in obese patients might be expected to further decrease $^{18}$F-FES uptake because of competitive binding. Paradoxically, however, higher body mass index correlated with increased tumor $^{18}$F-FES uptake in a retrospective study. Likewise, in a human dosimetry studies no notable uptake of $^{18}$F-FES in fat tissue was observed.

Ninety-eight percent of the circulating $^{18}$F-FES is bound to plasma carrier proteins and, therefore, its distribution volume is probably not affected by body mass in human beings. The higher tumor SUV seen in patients with high body-mass index is possibly a result of SUV adjustment for patients’ weight rather than an absolute increase.

**Liver function and metabolism**

Despite the rapid hepatic uptake and metabolism of $^{18}$F-FES, liver function is unlikely to
affect quantitative measurements of ER expression. Correction for individual differences in blood clearance and metabolites did not improve the correlation between $^{18}$F-FES uptake and semi-quantitative in vitro assays.\textsuperscript{10} In a second large retrospective study involving 278 patients with ER positive metastatic breast cancer, no association was found between the rate of $^{18}$F-FES metabolism and tumor $^{18}$F-FES uptake.\textsuperscript{40}

Concentrations of sex-hormone-binding globulin in serum

High SHBG concentrations decrease the bioavailability of free circulating $^{18}$F-FES. This is relevant because serum SHBG levels can vary greatly between patients, ranging from 2 nmol/L to 200 nmol/L.\textsuperscript{40} Moreover, treatments can lead to changes in SHBG concentrations in serum. SHBG production by the liver is increased by 40–50\% because of the estrogenic effects of this drug and decreased by 40–50\% because of the actions of aromatase inhibitors, but is unaffected by fulvestrant.\textsuperscript{76,92–94} Additionally, SHBG concentrations in serum are higher in patients with ER positive breast cancer than in those with ER negative tumors, and in lean patients than in obese patients.\textsuperscript{95} In a retrospective study based of 284 scans that assessed the association between tumor $^{18}$F-FES uptake and serum SHBG concentrations, 5–7\% reduction in $^{18}$F-FES uptake was observed for every 1.5 nmol/L increase in the square root of SHBG.\textsuperscript{40} No biopsy data were available, however so whether correction for SHBG levels would result in improved quantification of ER density is unclear.

CONCLUSION

Targeted therapies and personalized patient management of patients are rapidly emerging elements of breast cancer treatment. The classical point of view is to assume the characteristics of metastases are similar to those of the primary tumor, but evidence is increasingly pointing to changes in tumor phenotype and behavior during disease progression. Molecular imaging of characteristics that might be suitable treatment targets, such as ER, has the advantages of being non-invasive, providing quantitative information on all tumor lesions, and assessing changes before during and after treatments. $^{18}$F-FES-PET has clear potential to improve therapeutic decision making. Quantitative measurements of $^{18}$F-$^{18}$F-FES uptake reflect the availability of ER binding sites and, therefore, might improve prediction of response to endocrine therapy compared to standard immunohistochemistry. Validation of quantitative measurements by modeling studies with arterial blood sampling for bound and free $^{18}$F-FES and metabolites will be crucial to ensure refinement and reliability of SUVs. Factors to take into account are body composition, binding of $^{18}$F-FES to carrier proteins, hepatic $^{18}$F-FES clearance, and competition between $^{18}$F-FES and endogenous ligands. $^{18}$F-FES-PET also deserves further exploration as a method for monitoring ER binding, which could improve dosing regimens, in vivo assessment of binding characteristics in new
targeted drugs, and prediction of therapy response. Whether the use of $^{18}$F-FES-PET lowers the costs related to invasive biopsies and avoids multiple imaging examinations should also be explored.

**SEARCH STRATEGY**

We searched PubMed and Scopus with one or more combinations of the following terms: “antihormonal”, “breast cancer”, “(o)estradiol”, “(o)estrogen receptor”, “(o)estrogen receptor alpha”, “(o)estrogen receptor beta”, “FES”, “fluoroestradiol”, “positron emission tomography”, “PET”, “pharmacokinetics”, “SHBG”, and “sex hormone binding globulin”. The search results were manually screened for relevance and the reference lists of selected articles were checked for additional literature. We selected papers written in English and published between January 1970 and May 2013. We searched ClinicalTrials.gov for continuing clinical trials, up to May 2013, with the search terms “FES PET” and “fluoroestradiol”.

**CONFLICT OF INTEREST**

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